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U. S. DEPARTMENT OF AGRICULTURE
OFFICE OF EXPERIMENT STATIONS

A. W. HARRIS, DIRECTOR

EXPERIMENT STATION BULLETIN No. 9

THE
FERMENTATIONS OF MILK

BY

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PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE

WASHINGTON
GOVERNMENT PRINTING OFFICE
1892

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LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
OFFICE OF EXPERIMENT STATIONS,
Washington, D. C., January 20, 1892.

SIR: I have the honor to transmit herewith for publication Experiment Station Bulletin No. 9 of this Office, containing a résumé of our present knowledge regarding the decomposition changes in milk under the influence of ferments and bacteria, with special reference to the practical application of this information to the needs of the dairy industry. This bulletin has been prepared by H. W. Conn, Ph. D., professor of biology in Wesleyan University, who has devoted much time and energy to the collection of literature on this subject, as well as to original researches which have given interesting and valuable results.

Respectfully,

A. W. HARRIS,
Director.

Hon. J. M. RUSK,
Secretary of Agriculture.

THE FERMENTATIONS OF MILK.

By H. W. CONN, PH. D.

COMPOSITION OF MILK.

In the following study the word fermentation is used in its broadest sense. It will include not only the fermentations, commonly so called, produced by yeasts and rennet, but also all of the numerous destructive changes to which milk is subjected through the action of both organized and unorganized ferments. As we shall use the word, therefore, it will cover all of the processes of curdling, acid formation, putrefaction, etc., and in short all of the changes which may occur in milk after it is drawn from the cow.

It will be necessary at the outset to notice briefly some of the general facts known in regard to the chemical composition of milk, since upon its composition all of the fermentative changes are based. The composition of milk, however, varies widely with different cows and different conditions surrounding the cows, but a tolerably fair average composition may be given as follows: Water 87, fat 3.6, casein 3.3, albumen 0.7, milk sugar 4.7, and ash 0.7 per cent.

The solids represented in the above analysis are partly in solution, partly in a state of semisolution, and partly in suspension. Milk when freshly drawn from the cow is a very limpid liquid, but it becomes slightly viscous after standing for a short time, through the formation in it of a small amount of fibrin (Babcock [89]*).

The milk *fat* is usually said to consist of a mixture of several fats, most prominent among which are olein, stearin, palmatin, butyrin, caproin, caprylin, and rutin. To what extent these various fats are present in fresh milk, can not be definitely stated at present. Some of them are certainly not present in the milk when first drawn, but are due to decomposition changes which occur subsequent to the milking, either those of fermentation or oxidation. Fresh milk, for instance, has been shown not to contain butyrin, caprylin, or caproin, but is said to contain butin, meryslin, stearin, and palmatin. The composition of the milk fat seems to begin to undergo changes almost immediately after the milk

*The numbers in brackets refer to the list of references at the end of this bulletin and at the same time give the date of the publication of the article referred to.

is drawn, and its exact condition at any moment will therefore be very uncertain. The fat in the milk is in a condition of minute globules, varying somewhat in size and held in a state of a permanent emulsion. It was formerly believed that there was an albuminous membrane around the globules which prevented their ready fusion with each other. This belief has been abandoned at the present time, and it is now believed that the slight surface tension exerted by the difference in density between the fat and the albuminous liquid in which it floats, is sufficient to explain the reason why the globules do not readily fuse. Babcock [89] also thinks that his fibrin assists in the permanency of the emulsion. The white color of the milk has usually been attributed to the emulsified condition of the fat, but it seems probable that the calcium phosphate in the milk also contributes to the whiteness (see Conn [90]).

In regard to the nature of the *casein* there has been much discussion. In the first place it seems certain that the casein does not exist in the milk in a state of complete solution, but rather in the form of finely divided particles, or perhaps as a sort of colloidal gum. Probably some of it is in actual solution, while a greater part of it is in a state of suspension. When milk is filtered through porcelain, the casein, together with the fat, is filtered out, and a clear liquid called milk serum passes through the filter. In this serum there is a certain amount of albumen and usually also part of the casein which has been in solution in the milk, but the fact that the bulk of the casein does not filter tells us that it is not in a state of complete solution. The relation of the soluble and insoluble portions of the casein is a matter of much importance in the study of milk fermentations. The chemical nature of casein is not positively known. There seems to be considerable evidence for regarding it as quite similar to alkali albumen. A solution of alkali albumen will ordinarily filter through porcelain, but will not do so if mixed with butter fat. But while in many respects the chemical properties of casein and alkali albumen agree, there are some important differences between them. The former is precipitated by rennet, while the latter is not. The two can not, therefore, be regarded as identical. The important character of casein, upon which the manipulation of milk depends, is the readiness with which it is precipitated from its semisolution. The casein is readily thrown down from its solution by rennet, and also by a considerable variety of chemicals, among which are lead acetate, cupric sulphate, alum, mercuric chloride, tannic acid, sulphate of magnesium, and the mineral acids. It is not, however, precipitated by boiling, in which respect it differs from most proteids, which are coagulated by heat. The precipitation of the casein is commonly called curdling.

The milk *albumen* is in complete solution, and seems to differ only slightly from the serum albumen of the blood. Considering the intimate relation which the milk must have with the blood, or rather the

lymph, while in the mammary gland, it is not surprising to find one of its constituents so similar to this albumen of the blood. We may probably look upon it as derived directly from the albumen of the lymph. The presence of this serum albumen in the milk is perhaps an important factor in explaining the changes which Babcock has shown to take place in milk upon standing. If milk albumen is identical with serum albumen it is not surprising to find that a slight clotting should occur in the milk, from the formation of fibrin, similar to that taking place in blood. But we must remember that while milk albumen is very similar to serum albumen it differs in some respects, and this indicates that it is not simply filtered from the lymph, but is at least modified by the cells of the mammary gland. It has therefore received the separate name of *lactalbumen*.

There is some evidence that the amounts of fat, casein, and albumen are subject to changes after the milk has been standing for awhile. Some experiments have indicated that the casein may increase at the expense of the albumen, and that the fat may increase at the expense of the casein. These results have been denied, however, and at all events require confirmation in the light of modern knowledge of fermentation.

The milk *sugar* exists in the milk in a state of complete solution. Long-continued boiling of milk or heating it to a temperature above 100° C. frequently turns it to a brownish color. This was at first attributed to a caramelization of the milk sugar, but it has been recently denied by Duclaux.

The *ash* of milk contains potassium, sodium, calcium, iron and magnesium, in combination with chlorine, phosphoric and sulphuric acids, and other constituents. These are all in solution, unless it be some of the calcium compounds.

Besides the above-mentioned constituents there are a number of other compounds found in milk in minute quantities, but they have not as yet entered into the study of milk fermentations, and we may therefore at present neglect them. Milk is a very complex body, and a complete study of its fermentations will of course take into consideration all of its constituents. At the present time, however, all of our knowledge is confined to the study of the fermentations as affecting the milk sugar, the casein, the albumen, and the fat. We are at present evidently only on the threshold of study in this direction, and no one can tell us what may develop as we learn to include the action of ferments on the other constituents of milk.

FERMENTATION OF MILK BY RENNET.

It will be well for us to begin our study of milk fermentations with that form which has been known longest. Rennet is a preparation usually made from the stomach of a calf, and which has the power of precipitating the casein of milk in a very short time. The curdling of milk

by means of rennet has been practiced for many centuries. The first mention of cheese is in the Hebrew Scriptures by David, and the manufacture of this article has always been a well-known process. The curdling of milk by rennet is the only form of fermentation of milk known which is produced by an unorganized ferment. While there are many types of fermentation, all others, as we shall see, are brought about through the agency of microorganisms. This was not, however, understood until within comparatively recent years, for it has only been about thirty years since the organized and unorganized ferments have been clearly distinguished. It was very natural that the curdling of milk by rennet should be confounded with that produced in the ordinary souring of milk. No attempt was made to study either of the phenomena until the present century, and even after scientists had begun to experiment in this line the rennet action and the spontaneous souring were regarded as identical. When it was shown that ordinary sour milk was accompanied by a well-defined acid (lactic acid), it was supposed that the same acid was produced in milk by the action of rennet, and indeed all of the early observations pointed in this direction. The precipitated curd was in all cases found to be acid, due, as we now know, to the action of microorganisms, but supposed at first to be the result of rennet action. A large number of the early observers thought it possible to prove that the rennet converted the milk sugar into lactic acid and that the acid then curdled the milk. As early as 1840, however, Berzelius [40] distinguished the souring of milk from the rennet curdling. The question of the formation of an acid in the action of rennet was for a time regarded as one of great importance, and many observations were made for the purpose of settling the question. The results differed, however, with different observers, but little by little greater care in the observations began to show that the acid formed was only an accidental product. Selmi [46], Lehmann [50], and Heintz [53] showed that the action was independent of the formation of an acid, and Voelcker [61] found that rennet would curdle milk that had been rendered distinctly alkaline and the whey would be distinctly alkaline after the curdling. This discussion of the formation of an acid was only important at a time when no clear distinction had been drawn between the action of rennet and that of microorganisms. To-day it has ceased to have any significance, since we have so distinctly separated rennet action and the souring of milk, but the demonstration that rennet could act in an alkaline medium proved that rennet did not curdle milk by the formation of lactic acid from the milk sugar, as had often been claimed (Soxhlet [73]).

Further knowledge of the action of rennet has developed parallel with our knowledge of the nature of ferments in general. Liebig, in accordance with his general theory of fermentation, thought the action was due to the decomposition of albuminoids in the stomach membranes from which the rennet was prepared. This decomposition acted on

the milk sugar and produced lactic acid, which precipitated the casein. Hallier [67] attributed the action entirely to the organisms in the stomach, but meantime the distinction between ferments was beginning to be recognized. It was Schröder and Dusch [54] who first recognized that all fermentations were not alike in general characters. Some of the fermentative processes which they studied were checked by certain chemicals, like glycerin and alcohol, while others were not affected by these fluids. This was the first indication that ferments could be divided into two classes, but the authors failed to see the importance of their results. But later, after the masterly researches of Pasteur had revealed the agency of microorganisms in the production of certain types of fermentation, the distinction between the organized and unorganized ferments became clearer. It was soon shown by Soxhlet [73] that the rennet ferment was to be classed with the unorganized rather than the organized ferments, and Hammersten [72], the Swedish scientist, showed that a solution of pure pepsin had no power to curdle milk, thus proving that the gastric juice has more than a single ferment.

It was Hammersten who first undertook the study of rennet in such a manner as to give a clear idea of what the substance is. His researches lasted several years, and gave an account of this ferment, which has been but little changed up to the present day. In his first paper [72] he demonstrated at the outset that the action of rennet is entirely independent of the formation of an acid. The reaction does not change during the curdling, although the curd is usually acid from the action of microorganisms. He proved, secondly, that the action is entirely independent of milk sugar and affects the casein alone. He found that solutions of casein which had been entirely freed from sugar would curdle readily by means of the ferment. Now since, as had already been shown, the action of microorganisms in souring milk is on the milk sugar rather than the casein, these conclusions proved that the two processes were entirely different, the one acting on the milk sugar and the other on the casein, the one curdling the milk by the formation of an acid and the other not affecting its reaction. Hammersten also succeeded in separating the active principle of rennet from the other ferments associated with it in the gastric secretion. Dechamps, in 1840, had attempted to isolate this ferment and thought he had succeeded, giving the name *chymosin* to the substance. His isolation was not successful, however, but Hammersten did obtain the active principle of rennet in a comparatively pure state and entirely free from pepsin. For the ferment thus isolated he used the name of *lab*. With this pure ferment he made further studies, being able now to isolate its action from the confusing results usually associated with it from its impurities. This lab acts on casein and not on milk sugar nor albumen. The ferment was found by him in the stomach of a large number of animals. It was shown to be more abundant in young animals than in adults. As the young cease to depend so largely upon milk for

food, the production of this rennet by the gastric glands decreases. In addition to pepsin and his lab, Hammersten thought he could obtain evidence of a third ferment associated with them in the gastric juice. This third ferment produced lactic acid from the milk sugar. It is quite probable that this acid production was due to bacteria action. At all events bacteria are numerous in the stomach, and no one has succeeded in getting any good evidence for the existence of another unorganized ferment in the stomach; it certainly has not been isolated.

Shortly after this there appeared a paper by Soxhlet [73] which reached different results. He also affirmed that the action might occur in milk which appeared to be alkaline, but concluded that the rennet acted solely upon the milk sugar, breaking it up into compounds, one of which precipitated the casein in much the same manner as in lactic fermentation. Indeed he thought that lactic acid was formed, though its presence was obscured. He failed to take into account the action of bacteria, and some of his results are to be attributed to their action. In the same year Heintz [73] denied the conclusion of Soxhlet, demonstrating that rennet has no effect on milk sugar. The great confusion of the results of different observers up to this time was doubtless due in large measure to their failure to isolate rigidly the ferment that they were trying to study. If rennet is studied while mixed with a varying amount of pepsin and with half a dozen species of bacteria, it is not to be expected that much uniformity in results can be obtained.

It was a second paper by Hammersten [74] that again advanced our knowledge of the action of rennet. In this paper he studied the nature of the chemical action of rennet on milk. Having first shown that the casein of milk is in a condition of finely divided particles, he proceeded to determine the differences between the curdling by rennet and that produced by acids. Both produced a precipitation of the casein or a part of it, but the curd was quite different in its chemical properties in the two cases. If the acid curd, *i. e.* the casein precipitated by acid, was dissolved in a little alkali and the solution neutralized, there resulted a solution of casein which was not precipitated by rennet; hence there was something present in the original milk which was necessary for the action of rennet, but which was not contained in the acid curd. To demonstrate this he added to the casein solution just mentioned the whey that was left after the acid curd had been removed from the milk. This whey, he argued, ought to contain whatever elements were wanting in the solution, and therefore ought to render the solution coagulable by rennet. He found that it did so. Further experimenting along the same line showed him that the lacking material was some form of calcium, probably its phosphate. It therefore followed that calcium is necessary for the formation of the rennet curd, and such curd contains a certain amount of this material either in its chemical composition or in mechanical mixture with it. The acid curd contains no calcium. He then concluded that the casein

was a solvent of the calcium salt, but does not keep it in complete solution, the partially dissolved calcium aiding in giving the white color to the milk. From further experiments he concluded that the action of rennet was a chemical one and consisted in splitting the casein into two parts, one of which is readily soluble and can be found in the whey after the precipitation, while the other is an insoluble proteid and appears as the precipitate or curd. The soluble portion is small in amount, while the insoluble portion is abundant. Finally, he determined that curd produced in milk by high temperatures (120°C.) was the same as that previously produced by rennet.

Later still, a third paper by Hammersten [77] appeared giving more details. He perfected his method of purifying the rennet curd from acids, which have a great tendency to adhere to it. He found also that other alkaline earths could take the place of the calcium in the curdling, and that other salts could replace the phosphates. Acid casein he found to differ from rennet casein by containing less calcium and being less soluble. Acid casein he found to have the reaction of the globulins and to be quite similar to nucleo albumen. The presence of calcium chloride greatly hastens the curdling by rennet, and sodium chloride and potassium chloride are favorable to it. In general, he thought that the curdling under the action of rennet was quite similar to the clotting of blood.

This work of Hammersten has generally been regarded as authoritative up to the present time, and it has been accepted that the action of rennet consists of a chemical splitting of the casein into soluble and insoluble portions. But although the fundamental nature of the process has not been very seriously questioned, quite a number of further details have been made out by later work. The ferment rennet has been found in other places besides the stomach of mammals. It has been found in birds by Roberts, in fishes by Bengner, and in a number of plants by Gorup, Baginsky, Lea, and Meyer. Duclaux and many others have found it produced by numerous bacteria. Whether the ferment found is identical in all of these cases remains to be proved. All that has been determined is that a ferment occurs, quite widely distributed in the animal and vegetable kingdoms, which has the power of precipitating the casein of milk in a manner very similar to true rennet.

Schreiner [77] found that boiling milk rendered it difficult to coagulate with rennet, and Mayer stated that heating it to 70°C. had the same effect. Schreiner determined that the amount of rennet required to curdle a lot of milk was dependent upon the amount of solids in the milk. Danielsky and Radenhausen [80] confirmed Hammersten's method of isolating rennet in a pure condition, and described experiments which indicated that the casein of milk consists of two proteids. One of them seems to be serum albumen, which is soluble, while the other is alkali albumen. Under the action of rennet all of the proteid becomes changed into alkali albumen, which is immediately precipitated as curd. This explanation was subsequently denied by Hammersten.

Two valuable papers by Mayer appeared next [80 and 82]. He experimented upon the conditions necessary for rennet action. Thirty degrees C. proved to be the optimum temperature, while 60° C. killed the ferment. The rapidity of the action varied with the relative amount of rennet and milk. Heat was found to be evolved during the action, and he concluded that the process was a continuous chemical change. The amount of fat had no influence upon the action, nor had a motion of the milk; but common salt hastened the curdling, while alkalis were said to delay it. Rennet was found to act more quickly if the milk was a little sour, but the result was probably due to a mixture of the action of rennet and acid. Mayer confirmed Hammersten's conclusion that the curd produced by acid was different from that produced by rennet, finding, among other differences, a greater amount of fat in rennet curd, although the amount of albuminoids was about the same. Finally, Mayer showed that the presence of bacteria was not necessary for the action of rennet (as had been claimed by Hallier), although their presence might hasten the action. If rennet was extracted from the calf's stomach and kept in the cold its action was slower than if it had been extracted and kept at a temperature at which bacteria could grow rapidly.

An attempt was next made by Eugling [85] and Schaffer [87] to determine more exactly the nature of the chemical changes occurring when casein is precipitated by rennet. Eugling thought that the proteid compound in milk which was acted on by rennet was casein tricalcium phosphate. This compound, he said, was destroyed by rennet, and an insoluble compound of casein calcium phosphate formed. Boiling milk, he said, caused the phosphoric acid to unite with the calcium of the casein and form an alkaline albuminate which is not coagulable, but such milk is again coagulable if its original chemical condition is restored. Schaffer also thought that in fresh milk the casein was in chemical union with calcium phosphate, forming a soluble compound which would become insoluble under the action of rennet. Boiled milk, he found, could be rendered coagulable if saturated with carbonic acid gas, which gas he believed was necessary for rennet action. Schaffer, however, thought that the precipitation by acid was the same as that by rennet, except in the end result. The albumen calcium compound, he said, was transformed into an insoluble compound with phosphoric acid, and in the presence of an excess of acid this union continued until all of the albumen was thus united with the acid. Under the action of rennet and carbonic acid gas the union was the same, though less complete.

The work of Duclaux [84] gave some new facts and tended to throw some doubt over the earlier results. He studied milk by means of the process of filtering through porcelain, and thus separated the whey from the solid and colloidal portions of the milk. By studying the serum filtered through these filters he did not find any increase in soluble albumen under the influence of rennet. The serum obtained from

fresh milk contained as much albumen as that obtained from milk which had been acted upon by rennet, and hence he denied the truth of the generally accepted belief that rennet separated casein into a soluble and insoluble portion. This new method of study by the porcelain filter is not so simple, however, as it would at first sight appear, and Duclaux was certainly led into certain forms of error. He even denied that calcium phosphate was concerned in the action. Without further proof we can hardly accept this refutation of Duclaux, especially since more recent results tend again to confirm the view of Hammersten. Perhaps the most striking result of the work of Duclaux [87] on rennet was in connection with the amount of the casein which could be obtained as a solid curd by the action of rennet. He stated that part of the casein was in a state of complete solution in the milk and could not be coagulated by rennet under any condition. This portion was irretrievably lost in the whey. But even of the remaining portion, which can be precipitated by rennet, part might be lost to the cheese maker through the action of bacteria. As we shall see in a later part of this paper, there are a large number of bacteria which have the power of digesting casein and rendering it very soluble. If these organisms are in the rennet or in the milk and are allowed to act upon the milk for any length of time, quite a little of the coagulable casein may be digested by their action and will then not be precipitated by the rennet. This portion will remain in the whey and of course be lost. The action of bacteria will thus actually diminish the amount of curd which can be obtained from milk and their presence in any quantity is therefore to be avoided by the cheese maker. But since, as we shall see later, the presence of bacteria is an absolutely essential factor to the ripening of cheese, the aim of the cheese maker should be turned toward hastening the process of curdling rather than toward removing the bacteria.

It is very evident that no satisfactory chemical explanation of the action of rennet had appeared at this time, and indeed even to-day the matter is still very obscure. A few more facts, however, have been discovered. Arthus and Pages [90] have published the results of some interesting experiments, which indicate that there are two entirely different processes going on in milk under the action of rennet. As a result there are produced two distinct proteid substances, one coagulable at a temperature of 70° to 80° C. and easily precipitated by acids, while the other is coagulated only by a temperature of 95° to 100° C. and is not precipitated by acids. The first of these two proteids the authors name *caseogen*, and they find that this, in combination with the alkaline earths, forms the solid curd which appears after the action of rennet. The second proteid they call *hemicaseinalbumose*. This does not become coagulated by the alkaline earths and consequently remains in the whey after the rennet has precipitated the caseogen. These two proteids are each end products of the rennet action and not convertible the one into the other. No matter how long the rennet is allowed

to act the two products are found and it is therefore impossible to look upon one of them as a stage in the production of the other. It will be seen that according to these authors the rennet does not of itself possess the power of precipitating the casein of milk. The rennet simply changes its chemical nature and produces new compounds which are at first soluble, but which are soon precipitated by the calcium salts commonly present in the milk. If these are absent the precipitation does not occur.

Quite similar results have been reached by Halliburton [90] and Ringer [91]. According to these authors the casein of the milk (which they call *caseinogen* to distinguish it from the precipitated curd which they call *casein*) is completely precipitated by acid, but under the action of rennet undergoes chemical change. It seems to be converted into two proteid compounds. One of them is soon thrown down from solution by the calcium salts which are present, while the other is not coagulable and remains in solution in the whey. These two compounds would seem to correspond to the caseogen and hemicaseinalbumose of Arthus and Pages, and also to the insoluble and the soluble portion of the casein referred to by Duclaux. Furthermore it is evident that all of these results do, in essence, confirm the conclusion of Hamnersten reached sixteen years before.

Here the matter stands at the present time. The results are certainly as yet not very conclusive nor very satisfactory, but the following general summary may serve to bring together the conclusions which are to be drawn from the facts thus far advanced. The casein in milk exists in a peculiar condition, seeming to be in a semisolution or colloidal state, and while not rendering the milk noticeably viscous, it is still capable of being filtered out by the use of porcelain filters. The exact chemical nature of casein in whole milk is not known, but it appears to have a close relation to alkali albumen. To distinguish it from the curd it has been called *caseinogen*. This caseinogen appears to be kept in the condition of semisolution by the alkaline condition of the milk, for it is easily precipitated from the solution by the presence of a small quantity of acid. When thus precipitated it seems to be simply thrown from its solution without being altered in its nature. But the active principle of rennet has a very different effect upon it. Under the action of rennet the caseinogen is chemically changed. It is broken into two different proteids, one of which is easily coagulated, while the other is coagulated only with great difficulty. The former is readily thrown from its solution by calcium salts, and since these are always present in the milk, the result of rennet action is always to throw down the casein. This portion of the original caseinogen is then manufactured into the cheese, while the other portion, being soluble, goes into the whey and is lost to the cheese maker. The amount of protein thus lost may be still further increased through the action of bacteria, which have the power of digesting even the curdled casein, and this fact

teaches the advisability of using rennet in a manner which will produce the coagulation as quickly as possible. The rapidity of the action will depend upon the relative amount of rennet and the temperature, while it is delayed by alkalies and hastened by various salts.

The active principle of rennet is a chemical ferment or enzyme, which is distinct from the other digestive ferments in the stomach juices. It has been variously called rennet, lab, chymosin, and pixin. It seems to be somewhat widely distributed in nature among animals and plants, and it is a common product of bacteria growth. It is killed by a temperature of 70°C . and it acts best at about 35° . It is undoubtedly to be regarded as one of the digestive ferments.

Rennet, as we have just seen, is one of the unorganized ferments or enzymes. Of the numerous other forms of milk fermentation, some are undoubtedly produced by similar unorganized ferments, but the enzymes are themselves produced by the growth of microorganisms. Our best method of studying the matter will therefore be to turn our attention directly to the relation of microorganisms in milk.

THE SOURING OF MILK.

Naturally the first subject to claim our attention is the universal phenomenon of the souring of milk. It has for a long time been recognized that unusual and abnormal fermentations, such as blue milk and yellow milk, must be caused by something getting into the milk or the cow from the exterior to excite the trouble. The normal souring has, however, until quite recently been regarded as a characteristic of milk itself, unassisted by any extraneous influences. The universal occurrence of the souring of milk and the impossibility of preventing it by any of the common methods of preventing fermentation have been the cause of this belief, and it has required a great deal of evidence before scientists could be disabused of the notion that milk has a tendency toward spontaneous change. To-day there is such a uniformity of results on the part of all experimenters that it is no longer possible to question that the souring of milk is a fermentative process produced by organisms getting into the milk after the milking. The study of the fermentative changes in milk has been closely associated with the study of fermentation in general, and especially with the study of spontaneous generation. Milk is one of the liquids in which it is most difficult to prevent the growth of bacteria by the ordinary antiseptic methods. For this reason the final disproof of spontaneous generation was not possible until the fermentations of milk had been shown to be due to the growth of microorganisms.

The souring of milk is accompanied by the production of a certain amount of lactic acid. This acid was first extracted from whey by the Swedish chemist Scheele in 1780, but the first work leading to the modern study of milk fermentation was the chemical study of sour milk by Pelouze and Gay-Lussac [33] and the isolation of lactic acid as a

chemical compound possessing properties of a well-defined acid. Four years later Turpin [37 and 38], in the course of a long series of studies on fermentation, turned his attention to milk and concluded that the process of souring was due to something contained in the milk. This something he supposed to be obtained from the mammary gland of the cow and to be lodged in the fat globules. His microscopic study showed him that something sprouted from the fat globules and germinated into a sort of mold similar to if not identical with *Penicillium glaucum*. Similar growths he found in various dead bodies when exposed to the air, and he concluded that it was the presence of oxygen that caused the germs to develop. He was undoubtedly dealing with some of the numerous forms of molds which are so likely to appear in milk or other media suitable to their growth. The molds which he found probably came from the air, and, as we now know, had nothing to do with the souring of the milk.

At about the same time Schwann and Latour began to lay the foundation of our modern knowledge of the processes of fermentation, by claiming, on the basis of careful experiments, that fermentations are caused by living organisms. Schwann [37] performed the classic experiment of showing that air which had been heated would not produce fermentations in meat, while unheated air would do so. He further made a microscopic study of yeast and found that this body grew and multiplied during the fermentative process, and that all poisons which were capable of destroying the growth of organisms checked the growth of the yeast and at the same time prevented the fermentation. From all of this he concluded that fermentations and putrefactions were produced by the growth of organisms.

With this start in the way of a true explanation of fermentation in general, it became possible to understand the changes in milk. Long before (as early as 1701) Andry had discovered that sour milk contained living organisms. The observation was not fruitful in results and had been entirely forgotten. The first observation of more recent times was by Fuchs [41] who studied souring milk with the microscope and succeeded in finding two different organisms present in all cases. The organisms found were a micrococcus (which he called *Monas*) and a bacillus (*Infusor*). Beyond the fact of their presence, however, no conclusion was drawn, and the observation was interesting chiefly on account of being the first mention of bacteria in milk.

A step in advance was made by Blondeau [47], who confirmed Schwann's idea of fermentation by showing that each kind of fermentation was accompanied by an organism. In milk he found two of these organisms, though they seemed to be different from those found by Fuchs. One was a yeast (*Torula*) and the other a mold (*Penicillium*). Blondeau thought that the latter was the cause of the souring of milk, although he regarded the action as taking place through contact with

the milk rather than through the life of the organism. In 1852 we find Haubner concluding that the souring of milk was produced by something from the outside and not inherent in the milk itself. He thought it was thus as true an infection as that of blue milk, which had already been associated with microorganisms. In 1855 Schlossberger showed that milk would not sour in the mammary gland, even if allowed to remain there for a long time.

But these observations were isolated ones and at the time received little attention. The theory of fermentation advanced by Liebig had entirely captivated the minds of chemists and proved a great obstacle in the way of the acceptance of the agency of organisms in fermentation. Liebig supposed that fermentation was a property of all albuminoids. All albuminoids, he supposed, possessed a tendency to undergo decomposition changes and were thus ferments. We need not stop to consider this view, but the theory as advanced by Liebig soon won wide acceptance, and all attempts to find a vital explanation for fermentations were soon forgotten. The formation of lactic acid in souring milk having been shown to be due to a fermentation of the milk sugar, was now generally looked upon as a simple oxidation. Rowlandson [52] used this oxidation theory in the following ingenious manner: When cows run, he said, their respiration is faster; this of course causes them to absorb more oxygen, and the milk is therefore oxidized faster, becomes warmer, and sours more quickly. From our present knowledge of physiology and fermentation this conclusion of Rowlandson seems somewhat marvelous in its inaccuracies.

After a little a reaction took place against the views of Liebig, and the living factor in the fermentative processes began once more to be asserted. Schröder and Dusch [54] discovered the extremely important fact that a plug of cotton wool has the power of filtering microorganisms out of the air. This discovery proved of immense advantage in the study of microorganisms, for it served to simplify all experiments. With this new method of purifying air these authors soon discovered that two classes of fermentations could be distinguished, one caused by living organisms and the other caused by something which could not be removed by a filter and was not strictly alive. In other words, they distinguished between what we now know as organized and unorganized ferments. It was hardly to be supposed that they would understand the value of this discovery, which could only be appreciated after a better knowledge of microorganisms had been reached. In spite of their excellent work these authors were in error in regard to milk. Finding that they could not preserve milk by boiling it and then filtering all air that had subsequent access to it, they concluded that the souring of milk was a spontaneous change of milk itself.

It was not until the attention of Pasteur was turned to the subject that the theory of Liebig was finally disposed of and the true facts concerning milk were discovered. Between 1850 and 1860 he was carrying

on his classic experiments on spontaneous generation, which have led to such far-reaching results. He soon reached the conclusion that all fermentations and putrefactions were due to organisms introduced into the fermenting material either from the air or some other extraneous source. He was finally successful in establishing the fact that all fermentations are accompanied by microorganisms. For a time milk proved a stumbling block for it is not easily sterilized by heat, but he had no difficulty in showing that organisms were always present in fermented milk. In 1857 [57] he first described a special organism found in souring milk. He called this a yeast. His descriptions of the organism were of course of little value, since he had no means of obtaining a pure culture of it, but they were sufficient to show us that he had some of the species of bacteria which are found in similar places to-day and known to be the cause of the souring. His organism was doubtless a bacterium and not a yeast. That he did not have a pure culture of it is shown not only by his microscopic study, but also from the fact that in his fermented milk he found, in addition to lactic acid, several other products which do not result from the growth of the simple lactic organism. Later [58] he was successful in separating the lactic fermentation more distinctly from the alcoholic fermentation and made a more careful study of the lactic organism. He now claimed that the two processes were wholly distinct and that no lactic acid was formed in pure alcoholic fermentation [59]. Wherever lactic acid was found in fermenting material, there he claimed he could always find his lactic "yeast." This discovery really marked an era in the history of fermentation, for it was the beginning of the conception of a variety of organisms, each associated with a distinct form of fermentation. Up to this time there had been a tendency to regard all forms of fermentation as nearly alike, the differences shown depending upon the medium in which the fermentation occurred. With this discovery of Pasteur scientists began to see that perhaps there might be a variety among the forms of fermentation independent of the medium to be fermented. It took many subsequent years of experimenting to prove this, but the work of Pasteur was the first step in isolating distinct forms of fermentation accompanied by distinct species of organism. A later paper [60] dealt with the phenomena of milk which had been boiled and had subsequently fermented spontaneously. As already mentioned, Schröder and Dusch [54] had found that boiling would not prevent the fermentation of milk. Pasteur found the same, but he soon saw that such milk did not undergo the ordinary souring, but changed in an entirely different manner. It became bitter rather than sour and contained butyric rather than lactic acid. His microscope soon showed him that it contained numerous organisms, which were, however, quite different from those of sour milk [61]. These new organisms possessed a power of rapid motion and were regarded by him as infusoria. He thought that the organisms had been originally introduced into the

milk from the outside and had withstood the temperature of boiling to which such milk had been subjected. Acting upon this suggestion, he tried the effect of a greater temperature, heating the milk under a pressure of $1\frac{1}{2}$ atmospheres, and thus obtaining a temperature of 110° to 112° C. After such treatment he found that the milk would remain sweet indefinitely, with no other change than a slight oxidation of the fats. This of course still further convinced him of the importance of the agency of his newly found organism, and he soon [61] succeeded in separating it more completely from the lactic organism, and named it *Vibrio butyricus*. Butyric and lactic fermentations were thus clearly distinguished.

These results of Pasteur were not to go unchallenged, for the chemical theory of Liebig persisted in one form or another for some time. Hoppe-Seyler [59] concluded that while heating does destroy the power of milk to ferment, the power is restored again by the action of the oxygen of the air, and hence he supposed that the milk contained an already prepared ferment, which was destroyed by heat, but restored by oxygen. Later [81] he reaffirmed that the souring of milk is due to some unorganized ferment, and that this ferment is produced in the cow. In the later paper, however, he admitted that the microorganisms might hasten the process, since they produced the same ferment by their own growth. According to him, all fermentations, indeed, are caused by unorganized ferments (enzymes), but in some cases these enzymes are produced by bacteria. Hence, although bacteria may of themselves sour milk, he thought the milk might sour without their aid.

Schröder [61] studied the organisms found in milk and found certain forms which were not killed by a simple boiling, though they were by a long-continued boiling temperature. He found, further, that cooked milk would remain sweet when exposed to the air much longer than raw milk, and from this drew the conclusion that the organisms which produced the fermentation were derived from the cow. In this conclusion he overlooked the much greater chance which raw milk has had for contamination during the milking than the cooked milk, which was simply exposed to the air, and hence his experiment, though suggestive, did not justify his conclusion.

Trecul [72] several years later attempted to overthrow the whole fermentation theory of Pasteur. He identified Pasteur's lactic yeast with bacteria rather than yeasts, and then concluded that they were simply products of the albuminous matter of the milk. He thought they could be transformed into various species of bacteria and molds, and of course denied that they came from the air. His work has practically no value, since he took no precautions against contamination of his cultures, and all of his results had been previously refuted by Pasteur.

For several years Bechamp [72] very strongly advocated almost identical views. He insisted that the cause of the souring was in the milk itself, and he described minute living bodies (microzoma) in the milk,

which he insisted had been derived from the cow and were capable of developing into the organisms which had been described by Pasteur. For Bechamp, therefore, there were no such things as definite species of bacteria. The various forms described by Pasteur and others were only the forms which the microzoma had assumed under the various conditions. Bechamp for a time vigorously opposed the advancing views of Pasteur on fermentation, but the imperfection of his methods, compared with those of Pasteur, rendered his work valueless. The views of Bechamp and Trecul were little more than a revival of the old ideas of Turpin under a new name.

The work of Schmidt [74], though reaching a wrong conclusion, was of more real value. He found microörganisms in sour milk, but did not think them numerous enough to produce all the results attributed to them. He was, therefore, inclined to believe that there was from the outset a chemical ferment in the milk which produced the souring. He failed in his experiments to take sufficient precautions against contamination from the air, and his results are therefore untrustworthy so far as general conclusions are concerned. He did, however, show conclusively that milk sugar was a necessity for the curdling of sour milk and his study of the bacteria was a valuable one, although he failed to give them their proper share in milk fermentations.

A paper by Billroth in 1877 again asserted that milk was soured by a chemical ferment, but this was the last publication which denied the immediate causal connection between bacteria and the process of milk souring. The numerous experimenters in the fifteen years following Pasteur's work produced so much evidence in favor of the agency of microorganisms in producing all fermentations that the conviction soon became inevitable that Pasteur was correct in his conclusions. Let us now notice the various experimenters who contributed to this result, so far as milk is concerned.

First we may notice Van den Broek [60], who early confirmed Pasteur's conclusions that all fermentations were due to something in the air. He concluded that this something was not oxygen, but microörganisms. His work, while adding little to the results which Pasteur had reached so satisfactorily, confirmed them in such a manner as to make a valuable addition to the slowly growing views as to the agency of bacteria. In connection with other work on milk, Broek determined that ozone has no power to sour milk, a conclusion which has been reaffirmed and denied several times since.

Hallier [67] found several organisms in milk which he thought were the cause of milk souring, though he believed they were derived from the milk gland. Among them he found a species of *Penicillium*, a yeast, a species of *Oidium*, and a micrococcus, which latter form he regarded as a spore of one of the other organisms. Hallier [67] indeed regarded all bacteria and yeast as spores or stages in the development of the larger fungi and algæ.

Hoffmann [69] described two species of organisms found in sour milk, one a stationary and the other a motile form. He concluded that the motionless form was the cause of the souring and identified Pasteur's lactic yeast with the motionless species found by himself. This he thought was derived from the air. He for the first time introduced the word bacteria as distinct from yeasts and molds, and regarded the lactic organism as a bacterium rather than a yeast.

Harz in 1871 also found several organisms in milk before souring and concluded that they were the cause of the souring.

The next contribution to the subject was from the masterly hand of Lister. His first paper [73] gave a long and laborious method of inoculating milk in such a manner as to avoid the possibility of contamination from the air, and with this more certain method he studied several forms of milk fermentation. He used Pasteur's plan of sterilizing milk under a high heat. He found in milk several forms of bacteria which he regarded, however, as different forms of the same species, and to the species thus found he gave the name *Bacterium lactis*. In a later article [77] he improved his methods of work and simplified them very much. In this paper also he describes a method of obtaining a pure culture of the bacteria with which he was working. His method was that of dilution, as follows: A little milk was diluted with a large quantity of sterilized water. After the dilution each of several test tubes containing a nutrient solution were inoculated with a single drop of the diluted milk, the endeavor being to dilute the milk to such an extent that not all of the test tubes should show any growth after inoculation. If some of these tubes remained without any growth it was safe to assume that the diluted milk contained less than a single bacterium to a drop, and hence no drop would contain more than one individual. The test tubes which showed a growth were therefore probably inoculated with a single bacterium and the culture which resulted must have been a pure one, since it came from a single bacterium. Working in this manner he succeeded in obtaining a pure culture of the lactic organism, the first pure culture of this organism which had been isolated and one of the first pure bacteria cultures of any kind. The organism thus isolated Lister carefully studied and described, and he soon found that the numerous forms which he had previously regarded as varieties of a single species were really independent species with no connection with each other. It thus appeared that while there was one species of bacteria which is normally present in milk and produced lactic acid, there were others which had different effects and whose study remained for the future. The lactic organism he determined was common around the dairy, but not common elsewhere in nature, not even in the barn. He found that sterilized milk if exposed to the air in different places, in his laboratory, in a barn, or in the open air, or if inoculated with water would ferment after a time, but would not undergo the lactic fermentation. The normal souring of

milk was rare except in milk which had come directly from the dairy. This somewhat surprising observation has since been confirmed [Conn], and hence the conclusion is forced upon us that the lactic organism is peculiar to the dairy, but not especially abundant elsewhere in nature.

Meantime Roberts [74] had put a final link in the chain of argument by proving that pure milk has no tendency to ferment until contaminated with bacteria. In the course of some experiments on spontaneous generation he succeeded in drawing milk from the cow without allowing it to become contaminated with atmospheric organisms. To do this he introduced a sterilized tube into the milk duct, passing it upward for some distance to avoid the bacteria that might be around the opening of the duct. Then by drawing the milk into sterilized glass vessels and closing them immediately to prevent the contamination from the air, he succeeded in keeping all organisms out of the milk. Such milk was not only sterile, but it would remain sweet and unchanged as long as he desired. After this observation there was no longer room to claim that the souring ferment was derived from the cow, and the conclusion was demonstrated that the normal souring of milk, like other fermentations, was caused by microorganisms derived from some external source. The observation of Roberts was confirmed by Lister [77] and by Meisner in 1882, and many other observers have reached the same result in later years. To-day careful experimenters have no difficulty in obtaining milk free from bacteria. Recourse to the sterilized tube in the milk duct is not necessary, extreme cleanliness being all that is requisite.

The work of Roberts and Lister practically settled the question of the causal connection between bacteria and the souring of milk. It may be well, however, to notice one or two indirect confirmations of the result. Bert showed in 1878 that by a pressure of several atmospheres the souring of milk could be prevented, though such pressure had no effect on the action of unorganized ferments. Meyer in 1881 milked six sterilized flasks full of milk from a cow whose teats had been carefully washed. All of the flasks eventually coagulated, although after varying lengths of time. Some of them were not affected for ten days. Meyer justly points out that such a result would be impossible if the milk contained a ferment when first drawn from the cow. Upon such a supposition all of the test tubes should behave alike. Meyer also found that bacteria would diffuse through parchment paper. Another experiment of his was of interest: He filtered some sour whey through double thicknesses of filter paper and inoculated sterilized milk with the filtrate. Souring took place in the inoculated milk quite slowly, and this convinced him that the organisms act through growth and not by the production of an enzyme. An enzyme if formed would of course have readily filtered through the filter paper and should have acted rapidly.

In spite of these results several attempts were made to deny the truth of Pasteur's conclusions even after their demonstration. Bohlendorf in 1880 finding that a temperature of 60° C. would not prevent the souring of milk, concluded that it must be caused by an unorganized ferment. He found bacteria in abundance, but denied that they were directly concerned in the souring, although perhaps acting through the production of an unorganized ferment. Hoppe-Seyler [81], as we have seen, even in 1881 insisted that there was an unorganized ferment derived from the cow. Hagemann [82] concluded that from some source a chemical ferment is formed which produced lactic acid from the milk sugar, basing his conclusion upon the alleged fact that a solution of milk sugar would give rise to more lactic acid if inoculated with a considerable quantity of sour milk than if inoculated with a small quantity.

This last experiment of Hagemann reminds us of some recent work of Fokker's, which though occurring many years later, may perhaps be best considered here. Fokker [89] again endeavored to throw doubt over the question of the relation of bacteria to milk by appealing to a new set of facts. He did not question the results of his predecessors, and recognized that bacteria are necessary for the fermentative changes of milk, but he argued that the bacteria serve only as a stimulus, the real fermentative substance being in the milk itself. He looked upon the souring of milk as analogous to the clotting of blood, being a property inherent in it by virtue of its constituents, but one which does not exhibit itself except under certain conditions. It is the casein that is the active agent in the process, and he based his conclusion mainly upon the fact that the amount of acid produced in a given quantity of milk is dependent upon the amount of casein present rather than the number of bacteria. He argued that if the bacteria were the active agents the amount of acid should be proportional to their numbers. The real fermentative power, he claimed, was in the casein, and the only relation that the bacteria have to the process is as a stimulus, just as the power of muscle contraction resides in the muscle substance, the nerve stimulus only serving to bring the latent powers of the muscles into activity. The nerve stimulus is the exciting cause, though it does not supply the energy, and so the microorganisms serve as a stimulus to the real fermentative power of the casein. It is plain that this view of the matter shows a great similarity to the old idea of Liebig, which scientists had supposed was disposed of. Like the fermentative theory of Liebig, it regards the foundation of the fermentation to lie in the unstable condition of the casein.

A reply has been given by Scholl [90] to this argument of Fokker's. Scholl insists that the experiments do not justify the conclusions. He finds that the amount of acid formed is dependent upon the amount of food present for the bacteria to feed upon, and claims that all of Fokker's results are explainable by such an interpretation. A further reply has been given by Kabrbel [90], who calls attention to the fact that

the development of an acid in milk checks the further growth of bacteria. Kabrhel describes experiments of his own which show that casein possesses the power of uniting with a certain amount of acid and thus rendering it neutral or inert. If this is so, the more casein there is present the greater will be the amount of acid that can be neutralized, and hence the greater the amount that can be produced before it becomes strong enough to check bacteria growth. This is at least as natural an explanation of the experiments of Fokker as the one which he gives himself.

It would seem, therefore, that although we must still look upon the organisms as the active agents in fermentation, it is well for us to remember that the whole subject of the source of fermentative energy is as yet very obscure. There is no satisfactory theory of fermentation. Scientists have proved that microorganisms are frequently necessary to the process, but the manner in which they act is by no means clear. In some cases they certainly produce an unorganized chemical ferment, which is to be regarded as the direct agent in the fermentation (see page 46). In other forms of organisms there is good reason for believing that no such enzyme is produced, and even where it is produced we are just as much at a loss to explain how the enzymes give rise to their fermenting effect. We can not settle this question to-day, but leaving aside this theoretical question of the ultimate source of fermentative energy, we may regard it as definitely settled that all fermentative processes in milk, except that of rennet, are either produced or excited by the *presence and growth of microorganisms which get into the milk subsequent to the milking*. Without their presence, normal milk will remain sweet indefinitely. They are not present in the milk while in the mammary gland of the healthy cow, and their source is always an external one. If the cow should become diseased of course the matter is different. Milk from diseased cows undoubtedly may contain bacteria before it is drawn, and might of course ferment without contamination from without.

It should be noticed in this connection that there have been known instances in which milk has undergone what has been termed "spontaneous curdling." Such curdling takes place very quickly after the milk is drawn; in too short a time indeed for us to suppose that it could have been produced by bacterial growth. This phenomenon was first described by Hoppe-Seyler, and was subsequently denied by Lister [73]. Recently Levy [87] has insisted that such spontaneous curdling does occur. The milk, he tells us, becomes acid and curdles quickly, although there are no microbes in it, as he showed by microscopic study and culture tests. He regards the curdling in these cases as due to the acid formed by the death of the colostrum bodies of the milk. Milk certainly may become slightly acid through the decomposition of the tissue of the mammary gland, and perhaps this is the real explanation of the spontaneous curdling. Recently, however, Babcock

[89] has offered another explanation. He thinks, as we have seen, that there is always formed in milk shortly after milking a minute amount of fibrin, which renders the milk slightly viscous. This, according to him, is a normal constituent of milk. Ordinarily the milk does not become gelatinous enough to be noticeable except by microscopic tests, but he suggests that under certain abnormal conditions there may be enough of this fibrin formed to produce a clot in the milk identical with the clotting of the blood. It is too early to draw any conclusion in regard to this matter. We know almost nothing in regard to this fibrin constituent of the milk and scarcely more in regard to the so-called spontaneous curdling. Further study is needed in both directions before anything definite can be stated.

We will now return to the study of the lactic organism, which it will be remembered was first definitely isolated by Lister in 1873. After the publication of Lister's work experiments began to multiply and the knowledge on the subject rapidly increased. Boutroux [78] studied the lactic organism, which, however, he thought to be the same as *Mycoderma aceti*, and found that its growth was dependent upon the presence of oxygen, for when it is cultivated in closed flasks oxygen is absorbed and carbonic acid given off. He also first determined the important fact that the growth of the organism is checked by the presence of any considerable quantity of acid, and its growth in milk for this reason soon ceases because of the accumulation of lactic acid. Richet [79] concluded from the fact that the lactic organism grows most rapidly on the surface of liquids, that the rapidity of its growth is dependent upon the amount of oxygen. He also determined the relation of the lactic fermentation to temperature, finding that its growth increased up to a temperature of 40° and then diminished, ceasing entirely at 52° C.

The next important work was that of Hueppe [84], whose study of the lactic organism was the most complete that has ever appeared. By the help of modern bacteriological methods he was able to make a more thorough investigation of the bacteria of milk than had been hitherto possible. He isolated a number of bacteria from milk, the most common of which was one agreeing, so far as could be determined, with the *Bacterium lactis* of Lister. This organism Hueppe named *Bacillus acidi lactici*. He cultivated it in various culture media, determined its relation to temperature, and studied its effect on milk with great care. This lactic organism was found to produce no spores and was consequently killed by a moderate temperature. Hueppe applied Tyndall's method of discontinuous heating to the sterilization of milk, and found that a short boiling on three successive days was sufficient to sterilize completely. Since a lower temperature would kill the lactic organism he found that a temperature of 70° C. would also produce sterilization if continued long enough. It required five days to sterilize the milk at a temperature of 70° C., the milk being heated to this temperature for a short time each day. The lactic organism he found to grow best at a

temperature between 35° and 42°, and to be checked by a temperature over 42° C. When growing under normal conditions it curdled sterilized milk in twenty-three to twenty-four hours, producing lactic acid and carbonic acid. Hueppe also determined anew its relation to oxygen.

A second paper by Hueppe [84] gave a further discussion of the matter, and showed that the *Bacillus acidi lactici* was not the only species of bacteria capable of souring milk. He described five different species which he had isolated from milk, all capable of curdling milk and rendering it acid.

By this work of Hueppe the lactic organism was for the first time so clearly described as to be easily recognized by others. Although Lister had doubtless obtained a pure culture, he was unable to accurately describe it, owing to the imperfect bacteriological methods. After Hueppe's paper appeared, however, others attempted to find the organism in milk. Maddox [85], while studying the species, noted and described some abnormal forms. Beyer [86] studied sour milk in this country in order to determine if the same species produces the phenomena here as in Europe. He isolated a bacterium which was undoubtedly a cause of sour milk, and which he supposed to be identical with that described by Hueppe. His work was not sufficient, however, to prove the identity of the two species, although undoubtedly his organism showed close relationship to the *Bacillus acidi lactici*.

Marpmann [86] soon confirmed the conclusion of Hueppe, that more than a single organism may be concerned in the souring of milk. He found five species of bacteria in some specimens of milk studied by him, all of which curdled milk, four of them easily and the other only after a longer time. One of the five was identical with the lactic organism of Hueppe. All of his species formed an acid, and all grew most readily in the presence of oxygen, though they could also grow in its absence. When growing without oxygen, however, they formed no lactic acid. They behaved very differently in relation to heat; two of them were killed by an hour's boiling, two were not killed but greatly weakened by this treatment, while the fifth was not especially injured. None of his organisms formed volatile acids, and none of them were pathogenic.

The work of Hueppe and Marpmann was soon followed by numerous investigations on milk. Bacteriologists began to cultivate various species of bacteria in milk, using this as one of the means for separating species. Soon it became evident that the power of breaking up milk sugar and forming lactic acid from it was quite a common property of bacteria. Flügge [86] in his *Microorganismen* mentioned some fifteen species known to possess this power, and many others have been observed since by various experimenters (Grotenfelt, Baginsky, Kreuger, Conn, Clauss, Storch, *et al.*). Many other species have been found to produce an acid, but not enough to curdle the milk. But while the production of lactic acid has thus been shown to be quite a common

characteristic of bacteria, it does not appear that the action is the same in all cases. In only a few instances has it been definitely determined that lactic acid has been produced, and in some cases, at all events, other acids (acetic and formic) have been detected. Moreover, in the by-products accompanying the lactic acid there is wide variation among the several species, and some have been shown not to produce carbonic acid, while others certainly do. But there are undoubtedly many species of bacteria which, like the *Bacillus acidi lactici*, break up milk sugar and give rise to lactic acid. We have thus finally come to look upon the production of lactic acid from milk sugar as a form of fermentation due not to any specific organism, but rather to a class of organisms. Milk sugar seems to be ready to form lactic acid, and quite a large variety of organisms may be the exciting cause. Even in the dairy it seems that it is not always the same species of bacteria which sours milk. The lactic organism of Hueppe has certainly been found in many specimens of milk in Europe, though not by any means universally found. The lactic organism common at Wiesbaden is different from the common form in Groningen, one being a bacillus and the other a coccus (Fokker). In this country thus far no one has definitely found the *Bacillus acidi lactici* of Hueppe, or at least no careful description has been given of any species found here which enables us to determine with certainty that it is the same as the European organism. Other species, quite different from the organism of Hueppe, have been found to be the most abundant in souring milk, and it is certain that our most common lactic organisms are not the same as *Bacillus acidi lactici*.

The formation of lactic acid is thus the action of a class rather than any specific organism. But it is doubtful whether any two of the organisms of the class act on milk in precisely the same way. Indications strongly point to the conclusion that the methods of decomposition of milk vary widely. Haydruck [87] found that as lactic acid accumulates in the milk its production was hindered, but a microscopic study at the same time showed that the development of acid was not parallel to the growth of the organisms. At first the bacteria increased with the increase of the acid, but finally they ceased to grow, although the production of acid continued for some time longer. He concluded from this that the acid checked the growth of the bacteria but not their power of fermenting milk sugar, and hence that the fermentative power is something distinct from the growth of the organisms. Haydruck did not work with pure cultures and hence his work requires confirmation, but it does tell us at all events that we do not yet understand the process of lactic acid formation in milk. Warrington [88] found by quantitative tests that the amount of acid produced by different species of bacteria varies widely. Conn [89] has shown that many of the acid-forming species do not produce enough acid to curdle the casein. Baginsky [89] has found that one species studied by him produced acetone and acetic acid, together with hydrogen, carbonic acid, nitrogen,

and marsh gas. Nencki [91] found that the lactic acid produced by different species of bacteria is of a different chemical form. He even determined that two species which seem to be alike in every other respect may differ from each other in the chemical nature of the lactic acid formed. An observation by Grotenfelt [89] has shown that *Bacillus acidilactici* loses its power of producing lactic acid if it be cultivated for a long time in a sugar-free medium, although all of its other powers are retained. All of this indicates that the lactic fermentation of milk sugar is still a problem for solution, and although the causal connection between the fermentation and microorganisms has been conclusively demonstrated, the details of the process remain to be worked out. It is certain that the souring of milk is caused by bacteria, but it is also certain that quite a variety of species may under normal conditions be the exciting cause, and the satisfactory explanation of the manner in which the organisms work, whether by chemical or physiological processes, or both, is yet to be given.

NUMBER OF BACTERIA IN MILK.

After thus seeing that milk under all normal conditions will sour from the action of bacteria, the question of the number of these bacteria naturally suggests itself. Until Koch had invented the gelatin method of bacteriological investigation there was no reliable method of determining numbers. In recent years many quantitative estimates of bacteria in milk have been made. When we remember that milk while in the mammary gland is free from bacteria, we are hardly prepared to anticipate the immense numbers that are found in milk immediately after it is drawn. Cnopf and Escherich [90] found from 60,000 to 100,000 bacteria per cubic centimeter in milk a few minutes after the milking; Miquel [90] found from 10,000 to 20,000. Much smaller numbers than these have been found, for in reality the number is subject to the widest variation. These organisms all get into the milk from external sources, such as the hands of the milker, the air, the hairs or unclean udders of the cow, and especially from the vessels into which the milk is drawn, and plainly the number present in the milk will vary with the cleanliness used in the dairy and barn. By simply washing the cow's teats, drawing the milk carefully into a sterilized test tube and closing immediately to prevent the access of unfiltered air, it is easy to get milk so free from bacteria that it will remain unaffected for two weeks, even though kept all the time in a warm oven. In such cases the number of bacteria is of course very small. Indeed, as we have seen, it is possible to get fresh milk absolutely free from them. It follows therefore that the number of bacteria in milk immediately after the milking will vary from zero to many thousands, according to the conditions under which the milk is drawn.

With such numbers to start with we are prepared for almost any number after the milk has stood for awhile. Cnopf tells us that in cows' milk that is six hours old there may be from 2,000,000 to 6,000,000

bacteria per cubic centimeter. Of course the temperature at which the milk is kept will greatly affect the numbers. Conn [89] has found that in a specimen of milk kept for four days in a cold cupboard there were only 10,000 bacteria per cubic centimeter, while the same milk taken from the cupboard and left in a warm room for six hours contained 1,000,000 per cubic centimeter. Cnopf [89] has more recently studied the relation of temperature to the number of bacteria, comparing the rates of bacteria growth in milk at different temperatures. The following table indicates the results:

Rapidity of increase of bacteria in milk.

	Increase in number of bacteria —	
	At 34° C.	At 12.5° C.
In 1 hour	7½ fold.	None.
In 2 hours	23 fold.	4 fold.
In 3 hours	64 fold.	6 fold.
In 4 hours	215 fold.	8 fold.
In 5 hours	1,830 fold.	26 fold.
In 6 hours	3,800 fold.	435 fold.

This table shows in a marked manner the value of keeping milk cool, even to the moderately low temperature of 12.5° C. Miquel has found 100,000 bacteria per cubic centimeter in milk which has stood for fifteen hours at 15° C., and 72,000,000 per cubic centimeter in a similar lot which had been kept at 25° C., while milk kept at the temperature of 35° C. contained 165,000,000 per cubic centimeter. Freudenreich [90] found after twenty-four hours at 25° C. 577,500,000, while at 35° C. only 50,000,000 per cubic centimeter were present.

Such numbers as these are too large to make any adequate impression upon us and so large that the variations among them cease to have any special significance. It is rare that milk reaches the consumer with a bacteria content which can be measured in smaller numbers than tens of thousands, and frequently it will reach hundreds of thousands per cubic centimeter. By the time that it begins to become sour the numbers are much greater. These numbers are a striking commentary upon the bacteriological study of drinking-water. Drinking-water which contains more than 1,000 bacteria per cubic centimeter is usually regarded as unsafe, while milk containing as many as 1,000,000 is frequently used as food without hesitation and without any evil results. It is evidently not the numbers of bacteria which render milk or water dangerous, but the kind of species present.

It is a suggestive fact that the amount of acid does not increase regularly with the number of bacteria. For some days the number of bacteria increases with regularity, but the amount of acid does not thus increase. Miquel [90] finds that the amount of acid may be less after twenty-four hours than it is earlier. The significance of this fact we

can not give with satisfaction to-day. Miquel thinks it is due to the conflict of the different species of the bacteria in the milk. Some of the species, as we shall soon see, produce an alkaline decomposition of milk. Of course the growth of such species will in a measure neutralize the effect of the acid-forming class. We may probably look upon the different varieties of organisms in the milk as having a constant struggle with each other. Cunningham [91] has shown how this struggle affects the growth of the cholera germs in milk. This conflict will vary with conditions. Some species grow best at a high temperature, others best at a lower temperature. Different specimens of milk will moreover contain an entirely different assortment of bacteria, and this will greatly affect the result of the conflict between them. The result of the growth of such a miscellaneous group of bacteria as we find in ordinary milk will hardly be alike in any two cases, even though the conditions seem to be precisely the same. We should only expect uniformity of results when sterilized milk is inoculated with pure cultures of bacteria.

Some very remarkable experiments bearing upon the relation of bacteria to milk have been recently described by Fokker [90]. It has been recently discovered that freshly drawn blood has some power as a germicide, a power residing in the serum, which disappears in a few hours after the blood is drawn and is destroyed by boiling. This fact of the germicidal power of blood serum has plainly an important bearing upon the power of the body in resisting disease, but its exact significance has not yet been determined. It appears now, as the result of Fokker's experiments, that a similar power is possessed by milk. Experimenting first upon goats' milk, he found that freshly drawn milk is a partial germicide. Freshly drawn milk was inoculated with a certain quantity of bacteria and at the same time a similar quantity of milk sterilized by boiling was inoculated in a like manner. The sterilized milk always curdled quicker than that which had been inoculated while fresh. This suggested the germicide power and led to further experiments. He inoculated freshly drawn milk with a known quantity of certain species of bacteria and then made quantitative examinations of the bacteria in the milk at short intervals for several hours. He found that the numbers of bacteria in the milk actually decreased for a few hours, but then began to increase again. Fokker experimented with two species of bacteria and found similar results in both cases. He found that heating the milk destroyed its germicide power and that it disappeared of itself after several hours, thus in all respects agreeing with blood serum.

This conclusion of Fokker is certainly a surprising one when we take into consideration the rapidity of bacteria multiplication which occurs in milk, as indicated in the previous pages. An increase of twenty-three-fold in three hours, as shown in the table given above, does not look as if there was much of a germicide power in the milk,

but still we are forced to accept the truth of Fokker's experiments, since they have been independently confirmed by Freudenreich [91]. Freudenreich confirmed all of Fokker's experiments and showed that the germicide power is associated with the serum of the milk. He experimented with a number of species of bacteria and found that the germicide power of the milk was not equal for all species.

Evidently this germicide power is intimately associated with that of the blood, and indeed since the milk serum contains an albumen that is closely related to serum albumen, it may be that the germicide power of the blood and of milk are really identical. Since Babcock has shown that fibrin is formed in milk like that in blood serum, and since we now find a similar germicide power associated with the two liquids, the great similarity of milk serum and blood serum is forcibly impressed upon us. While we can not regard milk serum and blood serum as identical, we must certainly look upon them as closely related in their albuminoids, and may regard milk serum as blood serum slightly modified by the mammary gland.

Of course this germicide power is of little practical importance so far as the preservation of milk is concerned. We have just seen that under normal conditions the common bacteria, of the dairy at least, multiply rapidly in milk and become very numerous after a few hours. So far as the practical side of this discovery is concerned, it only makes more evident the value of keeping milk as cool as possible from the very outset if we wish to avoid the troublesome growth of bacteria. Possibly this power of milk may be of some value in warding off disease and may be associated with the value of a milk diet in the case of certain intestinal germ diseases.

It remains to be noticed that the rapid multiplication of bacteria in milk does not continue for a very long time. For a day or two they increase with great rapidity, but then their multiplication is checked and finally they entirely cease to grow. This can not, of course, be due to a lack of food, for there is plenty of food in the milk at all times. It is rather to be attributed to the accumulation of the products of their action. Those which produce an acid will soon be checked by this, for bacteria can not grow in an acid medium. The amount of acid, however, will vary, for some species of bacteria are very sensitive to acid, while others will endure a larger amount without injury. Experiment has shown that different species vary widely in regard to the amount of acid which they will produce before their growth is checked. In regard to the bacteria which do not produce acid, the growth seems to continue for a longer time, but here, too, it is eventually stopped by the accumulation of the products of decomposition.

RELATION OF ELECTRICITY TO THE SOURING OF MILK.

The subject of the souring of milk would not be complete without reference to the effect of electricity. It is a popular belief that thunderstorms will sour milk, a belief so widespread that it would seem there

must be some foundation for it. It is very questionable, however, whether there is really any connection between the thunderstorm and the souring of milk. That souring frequently occurs during a thunderstorm, can not be doubted. As long ago as 1863 Henrici thought this was due to the formation of ozone by the electric shock and that the ozone hastened the souring. Van den Broek had previously denied that ozone had any such effect. Iles [77] performed some experiments which seemed to show that electricity will sour milk under conditions favoring the formation of ozone. In his experiments he placed the milk in eudiometers, together with pure oxygen. Electric sparks were then passed through the oxygen. He said the milk curdled rapidly and attributed it to the ozone formed. These conclusions were subsequently denied by Conn [90], who could not curdle milk by means of the electric discharge, even though abundant ozone was formed. Tolomei [90] claimed that the electric spark from a Holtz machine discharged at the surface of milk rendered it acid, although an electric current passed through it actually delayed the souring. In a subsequent paper [91], however, he reached different conclusions, claiming that neither the current nor the spark would sour the milk, but might, on the other hand, greatly delay the souring. At the same time he attributed the rapid souring during a thunderstorm to the ozone in the air.

Lastly Treadwell [91] has shown that the electric spark has no power to sour milk. He worked with both sterile milk and with normal milk from the dairy. He found that sterile milk was entirely unaffected by electricity and would remain for months unchanged after the electric spark had been discharged over its surface. The souring was wholly dependent upon bacteria and if these were not allowed access to the milk the electricity had no effect. Treadwell moreover experimented in such a manner as to produce abundant ozone, repeating Iles's experiments. But his results were different, for no marked effect was seen even when the ozone was abundant. With normal milk already containing numerous bacteria, he found that the electric shock did possibly hasten the souring very slightly. Milk without the electric shock curdled, for example, in twenty-four hours after the electric discharge, while milk treated to the electricity curdled in twenty-three hours. But evidently this slight increase in rapidity is not a factor of any great importance in milk souring. The slight hastening he thought might be due to ozone.

From these various experiments we must draw the conclusion that electricity is not of itself capable of souring milk or even of materially hastening the process. Nor can the ozone developed during the thunderstorm be looked upon as of any great importance. It seems probable that the connection between the thunderstorm and the souring of milk is one of a different character. Bacteria certainly grow most

rapidly in the warm, sultry conditions which usually precede a thunderstorm, and it will frequently happen that the thunderstorm and the souring occur together, not because the thunder has hastened the souring, but rather because the climatic conditions which have brought the storm have at the same time been such as to cause unusually rapid bacteria growth. Milk deprived of bacteria will certainly keep perfectly well during thunderstorms. Dairymen find no difficulty in keeping milk if it is cooled immediately after being drawn from the cow and kept cool. Milk submerged in cool water is not effected by thunder. Our dairymen find that during "dog-day" weather, even when there is no thunder, it is just as difficult to keep milk as it is during thunderstorms, and they also find that scrupulous cleanliness in regard to the milk vessels is the best possible remedy against souring during a thunderstorm. It is safe to conclude, therefore, that in all cases it is the bacteria which sour the milk, and if there seems to be a causal connection between the thunder and the souring it is an indirect one only. Climatic conditions have hastened bacteria growth and have also brought on the thunderstorm. The same conditions would affect the milk in exactly the same way even though no thunderstorm was produced, and this our dairymen tell us frequently happens during the warm, sultry autumn days.

OTHER FORMS OF FERMENTATION OF MILK.

It has not been until recent years that students have recognized that there is a great variety of fermentations liable to occur in milk. In all of the early work the observers who were trying to study the changes in milk failed to notice that they were not always studying the same thing. To them the spontaneous fermentation of milk always meant the same thing, except in a few special cases. It was seen that milk would ferment even after it had been boiled and excluded from contact with air, and it was taken for granted that the fermentation was identical with that occurring without such boiling, no one noticing that the fermentation that really occurred under these conditions was of an entirely different character from that of ordinary sour milk. If this had been clearly seen at the outset the question of the production of an unorganized ferment in the cow would have been settled in the negative long before it was. Slowly, however, it began to be seen that the fermentations of milk are somewhat varied. At the very outset blue milk and yellow milk were recognized as distinct forms of infection. Pasteur separated the butyric fermentation from lactic fermentation in 1860. Lister noticed several other forms of fermentation in 1873, and in later years the types of fermentative changes have been multiplied.

The reason for this slow rate of discovery is easily seen. Under ordinary conditions milk always undergoes some sort of lactic fermentation; only under rare conditions is this absent. The production of lactic acid soon curdles the milk and immediately obscures all other forms of

fermentation which have occurred simultaneously with it. The acid also stops the growth of all bacteria, so that no subsequent effect can be seen. Hence it is rare that we get in normal milk any palpable evidence of fermentation of any other sort than souring. But the study of bacteria from the time of Pasteur showed that there were a large number of different species of organisms, and while this view was for a time disputed it soon became definitely demonstrated. It became evident, therefore, that with a variety in the species of organisms a variety in the fermentations of milk could be expected. From the time when Koch invented the gelatin methods of study and of obtaining pure bacteria cultures with ease, investigations upon the effect of pure cultures upon milk began to be more fruitful in definite results. Our knowledge of the newly discovered fermentations is by no means complete as yet. We are indeed beginning to recognize that each species has probably its own distinct effect on milk. The matter thus becomes very complex, but although there is thus a great variety, still we find ourselves to-day able to divide the fermentations of milk into classes, each characterized by a single general action but comprising many varieties. The first class has been noticed, and its general character is the production of lactic acid. The second class is characterized by the production of an alkaline reaction instead of an acid.

ALKALINE FERMENTATION OF MILK.

That the fermentation of milk is not always accompanied by the production of an acid was first noticed by Haubner [52]. He found that a spontaneous change occurred in certain specimens of milk by which it was curdled but not rendered acid. He was convinced by experiment that the action which occurred was similar to that of rennet. Many of the early observers found that milk would undergo changes after boiling, and while these changes were frequently accompanied by a curdling, it became apparent after Pasteur's work that the changes occurring in boiled milk were very different from those of ordinary souring. By later work further details of the matter have been made out, and it has been ascertained that no lactic acid appears in spontaneously fermenting boiled milk. The milk may become coagulated into a soft or slimy coagulum which usually possesses a bitter taste. The taste is never sour, and the milk, instead of having an acid reaction, is either alkaline or neutral. After a day or two the coagulum begins to dissolve into a somewhat clear liquid, and if the action is allowed to continue long enough the curd may become completely dissolved into a semi-transparent liquid having no resemblance to milk. The chemical study of this liquid shows a variety of ingredients, among which are peptones, leucin, tyrosin, and ammonia. To the peptone the bitter taste may be at least partly attributed, and to the ammonia the alkaline reaction.

There is a large variety of forms of fermentation accompanied by the alkaline reaction, but three distinct features of the general class may

be conveniently selected for discussion. The formation of butyric acid, the formation of a bitter taste, and the curdling of the milk, with the subsequent digestion of the curd are all striking characters which may be considered separately.

BUTYRIC ACID.

The first mention of a butyric acid fermentation of milk was by Pelouze and Gelis [43]. These experimenters noticed that under what seemed to them to be similar conditions milk might undergo two different forms of fermentation, one giving rise to lactic acid and the other to butyric acid. They made a careful study of butyric acid although they had no idea of the reason why butyric acid was produced in some of their experiments and not in others. A similar confusion appeared in Berthelot's work for he found that under precisely similar conditions, as he supposed, it was impossible to tell whether an alcoholic or a butyric fermentation would ensue.

From this early time the matter was generally overlooked until, as already noticed, Pasteur succeeded in separating the lactic fermentation from the butyric fermentation. His paper of 1861 first raised the butyric fermentation to the rank of a distinct type and associated it with a distinct organism. Pasteur described an organism found in butyric fermentation, calling it *Vibrio butyrique*, regarding it as an infusorian and thus radically different from the lactic organism which he regarded as a yeast. The *Vibrio butyrique* of Pasteur was obtained from milk that had been boiled and subsequently allowed to ferment spontaneously. His cultures of it were of course not pure ones and his study not satisfactory to us to-day. But the work was of the utmost value since it was a departure in a new direction. Pasteur studied the organism enough to discover the very important fact that it grows best out of contact with air, and thus made at this time the discovery of the important class of anaërobic organisms.

As we have already seen, it was for a long time believed that the changes occurring in boiled milk indicated the presence of some unorganized ferment derived from the cow. This position was held long after Pasteur had shown that heat would sterilize milk, and even after Lister had shown that pure milk, without bacteria, had no tendency to change. The study of the butyric fermentation proceeded but slowly and all that was known about it for a number of years was comprised in Pasteur's conclusion that the fermentation was caused by a definite organism. But even this position was not accepted for several years. Hoffmann [69], in his valuable monograph, did indeed regard the butyric fermentation as due to special bacteria, but thought that it was simply a later stage in the process of the lactic fermentation and due to the later growth of the lactic organism. He did find a second form of bacteria present in milk undergoing butyric fermentation but thought that it was not present in sufficient abundance to be the cause of the production of the butyric acid.

The work of Pasteur was in part confirmed by Paschutin [74]. He proved that the butyric fermentation could be delayed by filtration, and from this result concluded that it was caused by a solid rather than a liquid body. He found that it could be almost prevented by a temperature of 50° C., although it would begin again after several days. He noticed also that glycerin and alcohol would prevent the fermentation, and from the fact that the unorganized ferments are not at all injured by these chemicals, he concluded that the fermentation must be produced by an organized ferment rather than an enzyme. He also found bacteria present in the fermenting liquids but gave no adequate description of them and was unable to decide whether they came from the cow or were introduced into the milk after the milking. This last point was of course soon settled by the classic experiments of Hall and Lister.

The distinct nature of the butyric fermentation was now fully recognized. Van Tieghem [79] determined its causal connection with a bacterium which he called *Bacillus amylobacter*, but which he soon recognized as identical with Pasteur's *Vibrio butyrique*. He even thought he could recognize this species preserved as a fossil in the Carboniferous rocks. This observation can not of course be regarded as having any value since we know to-day that the microscope alone is entirely inadequate to distinguish species of bacteria from each other.

Prazmowski [80] also made a careful study of the organism and used a new name, *Bacillus butyricus*. The species described under this name was an exquisite anaërobe, not growing in the presence of oxygen at all. In the absence of oxygen it grows rapidly and produces abundant spores, which have the power of resisting a high degree of heat. The presence of these spores explains the difficulty met by all observers who have tried to sterilize milk by heat. Boiling will not kill the spores of this butyric organism, and hence after boiling milk will be pretty sure to undergo the butyric fermentation. The lactic bacteria are at the same time killed, and hence the butyric fermentation is not obscured by an acid formation and curdling. *Bacillus butyricus* was found to produce a rapid decomposition of milk, considerable quantities of butyric acid being formed, with the evolution of carbonic acid and hydrogen. Its growth in normal milk is not very great for the first few days, nor indeed until the milk sugar has been largely turned into lactic acid by the ordinary lactic organisms. At first this seemed to indicate a necessity for the presence of lactic acid, but this is more than doubtful. The butyric species will certainly grow in sterilized milk, where there is no possibility of the production of lactic acid. The phenomenon seems rather to be due to the necessity of getting rid of the oxygen dissolved in the milk. The lactic organisms use this oxygen and then the anaërobic species can begin to grow.

After the work of Prazmowski others studied the butyric organisms and their descriptions were soon found to differ quite a little, until much

confusion arose. The names *Bacillus amylobacter* and *B. butyricus* have been used as applying sometimes to the same and sometimes to different organisms. A butyric acid organism of some kind was soon found to be very common. It was found in many specimens of old milk; it was found in the intestines of both sick and healthy persons; and also in fermenting cellulose. Fitz [82] described an organism which produced butyric acid and which he regarded as identical with Pasteur's organism, although it differed in some particulars. This organism did not curdle milk nor render it acid. Besides butyric acid, it produced as decomposition products, butyl alcohol, ethyl alcohol acetic acid, capronic acid, lactic acid, and succinic acid. It formed spores which resisted fifteen minutes boiling. Two years later Fitz [84] described a second butyric organism differing widely from the first. It did not produce spores and was killed by a temperature of 59° C., but it did produce butyric acid and acetic acid.

In this same year Hueppe [84] made a study of the butyric fermentation in connection with his work on the lactic organism. This species of bacterium, which he described as associated with butyric acid, was not strictly anaërobic, although he at first regarded it as identical with *Bacillus butyricus*. Still another species was soon described by Liborius.

By this time it had become evident that the butyric fermentation was to be regarded rather as a class fermentation than as due to any single species. Gruber [87] made a new study of boiled milk with the assistance of more recent methods and found more than a single species of organism. The bacteria in such milk, which had been formerly all united under one name, he found to consist of at least three different species, two of which were anaërobic while the other was not. All three could produce butyric acid and butyl alcohol. In the same year Loeffler [87] independently undertook a similar series of experiments and separated the so-called *Bacillus amylobacter* into four species, some aërobic and others anaërobic, and all producing different decomposition products of milk. Flügge [86] states that in his laboratory several species of bacteria have been found with the power of producing butyric acid. More recently other species have been found by Krügger, Conn, Lafar, and others, until we are finding that the butyric-acid class seems to be as large as the lactic-acid class of organisms. Some of them produce alkaline reactions, as we have seen, while others render the milk acid. The confusion which has arisen concerning the butyric organism has thus been removed as we learn that the action is a class fermentation rather than one associated with any distinct species of organism.

It will be best to introduce here a word as to the practical bearing of this butyric fermentation in the dairy. In the ordinary handling of milk this class of organisms is of little importance, but it has been supposed that they play an important part in the keeping properties of butter. Rancid butter contains considerable quantities of butyric acid,

and the development of the rancidity is parallel to the appearance of the butyric acid. Now, knowing that many species of bacteria do produce butyric acid and knowing that there are many bacteria in butter, it was a natural inference that the rancidity was produced by them. This seemed for a time in the line of experiment and especially along the line of our knowledge of fermentative processes in general. But it now appears very doubtful whether bacteria really have much to do with the phenomena. As far back as 1882 Hagemann [82] came to this conclusion, since he found that bacteria would not produce butyric acid from fats and milk sugar. He found further that rancidity was not contagious. The addition of lactic acid to fat would produce a butyric fermentation, while the inoculation with bacteria would not do so. His conclusion was that the butyric acid was formed from a glyceride, which latter compound was formed from the milk sugar by the action of lactic acid. Hence it followed that the rancidity of butter could be prevented by a thorough washing, which should remove the lactic acid, a method of little practical value, however, since it at the same time destroys the proper butter aroma.

The matter was further studied by Duclaux [86] with similar results. He also concluded that the rancidity was due to a slow decomposition of a glyceride, favored by the presence of water and some acid, but retarded by salts. During the process oxygen is absorbed without an equivalent elimination of carbonic acid; Duclaux determined, however, that the presence of bacteria may hasten the process. The bacteria act upon the glycerides and also upon the nitrogenous matter. If the amount of nitrogenous matter is small the butter becomes acid under the influence of the bacteria and the acid assists in the saponification of the fats. If the nitrogenous matter is greater in amount quite a different process occurs. At best, however, the bacteria only assist in the rancidity, but are not necessary for it. The development of rancidity in butter, according to Duclaux, is not a vital process. Indeed Duclaux finds that sunlight will produce decomposition effects very similar to those produced by organisms, and he institutes a close comparison between the fermentative changes proper and those which may be brought about simply by sunlight.

There seems to be no doubt that so far as butter is concerned these conclusions are essentially correct, for at least two observers have subsequently confirmed them. Ritsert [91], experimenting by isolating the bacteria from rancid butter and inoculating them into sterilized butter fat. No butyric acid was produced under the conditions, although examination showed that the bacteria had been growing and increasing in numbers. The same result was reached when sterile fat was inoculated with rancid fat, thus showing that the process was not of the nature of a fermentation. The rancidity he concluded was the result of the combined action of sunlight and oxygen and was thus in reality a true oxidation of the chemical sort. The exclusion of air was shown to be a preventive of rancidity.

These general results have still more recently been confirmed by Sigmund [91] and Lafar [91]. The latter finds that butter will not become rancid in a stream of hydrogen, although the bacteria may grow rapidly under these conditions. We must therefore conclude that while there is a large class of bacteria which produce butyric acid, their action is not necessarily connected with the formation of butyric acid in butter. Rancidity may be hastened by them, but it will occur in spite of their entire absence, and is probably of the nature of a direct chemical oxidation closely connected with the agency of sunlight.

Returning now to the study of the butyric organisms, we notice that the various organisms of this class, although all capable of producing butyric acid, differ widely from each other. So far as any further common property is concerned it is interesting to note that they all appear to grow best in the absence of oxygen, although not all of them are so dependent upon this condition as the original organism of Pasteur. But as for other properties they show the widest variation. It will be remembered that the general class of milk fermentations under which the butyric class has been grouped is distinguished by the presence of an alkaline reaction. It must not be understood however that the reaction of the butyric organisms is always alkaline; sometimes it is distinctly acid. Of course this alkaline reaction can not be due to the butyric acid, and must therefore be sought for in some of the other decomposition products.

But the study of the other chemical changes has thus far proved rather delusive because of their variety and complexity. From the first work of Pasteur it was seen that the formation of butyric acid was not the only effect of the fermentation, and many attempts have been made to study the other chemical changes. Meissl [82] made a chemical study of milk which had spontaneously fermented after boiling. After several weeks he found the milk had assumed a bitter taste, that the fat had collected on the surface, and that a yellowish scum had appeared with a granular precipitate. His analysis showed milk sugar, casein, peptone, leucin, tyrosin, ammonia, and a proteid midway between egg albumen and peptone. The fat had become rancid. At about the same time Löw [82] made a chemical study of some milk which had also spontaneously fermented after boiling, and had been standing for eight years. He found ingredients somewhat similar to those found by Meissl, although he found that all of the milk sugar had changed into lactose and glucose, and he found in addition considerable butyric acid.

Of course such chemical examinations as these have little significance to-day, since we have learned that the milk had not undergone a simple fermentation, but one produced by the combined action of a number of species of bacteria. But very little has been added to this side of the matter as yet, and the whole subject of the chemical side of butyric fermentation needs revision in the light of modern pure cultures of bacteria.

It may perhaps be stated that in all cases studied thus far it has been found possible to determine the presence of peptone in the fermented milk, and frequently the presence of leucin and tyrosin has also been determined. Lactic acid has been shown to be produced by certain species in connection with the butyric acid. But these matters belong to another division of the subject, and their consideration will be for the present postponed.

BITTER MILK.

Out of the general confusion which has surrounded the subject of butyric fermentation have gradually crystallized some definite ideas in regard to the phenomenon known as bitter milk. Pasteur noticed that milk which had fermented after being boiled became bitter, and the milk studied by Meissl and Löw had the same bitter taste. Now bitter milk is a well-known trouble to the dairyman, and it has been tacitly assumed that this trouble was associated with the growth of some butyric organism in milk. But this is certainly not always true, for other causes are sometimes at fault. The milk of old milch cows is said to have a tendency to be bitter, and various foods which the cow may eat are also thought to have the effect of producing bitter milk. Probably these and other causes may be occasionally at work, but in addition it is certain that microorganisms are frequently the cause of the trouble. Nägeli was the first to attribute bitter milk to the agency of bacteria and it has been a general observation that the fermentations which produce butyric acid are almost always accompanied by a bitter taste. This has led bacteriologists to ascribe the bitter taste to the production of butyric acid, and the common bitter milk has been ascribed to some butyric organism. No attempt was made to determine the exact relation of the butyric acid to the bitter taste until 1890. At that time Krügger [90], studying bitter milk, concluded that it might be due to several causes, one of which was the growth of bacteria. In the latter case he thought that the bitterness was actually due to the butyric acid which was produced, and he ascribed the trouble to the action of the bacteria species *Proteus vulgaris*. Soon after this Weigmann [90] questioned the conclusion, showing that butyric acid of itself has no bitter taste, and finding further that the bitter taste might be produced in milk by bacteria which do not produce any butyric acid. He found in a specimen of bitter milk a bacterium which produced the taste without any butyric acid, and thus proved that the bitterness was not a part of the butyric fermentation proper. Still more recently Conn [91] has shown that the bitter taste may undoubtedly be associated with butyric acid. He isolated from a lot of intensely bitter cream a micrococcus which rendered the milk very acid and very bitter, and at the same time produced butyric acid. This new observation was thus again in harmony with the previous work of Hueppe, Loeffler, and others.

From all of this we must conclude that bitter tastes of milk are undoubtedly at times produced by microorganisms. At times it is associated with the production of butyric acid, and at other times it is produced by organisms which do not produce butyric acid, but in all cases the bitterness seems to be independent of the acid. Evidently there are quite a number of species which produce this effect. Three have been described with care, and several others incidentally noticed, but we have no means of knowing how numerous they are. We have thus another class of organisms with a generic action on milk. We are not yet in position to say positively to what sort of decomposition products the bitter taste is due, but it seems most likely that it is associated with some of the products of albuminous decomposition. Hueppe [91] points out that the species producing the bitter taste all give rise to a decomposition of the albuminoids of the milk, producing peptones or similar products. Now peptone certainly has a bitter taste, and Hueppe thinks that the bitterness of milk is due to these albuminous products. This certainly seems the most probable explanation. At the same time it would seem that this is not the whole of the matter, for while, as we shall presently see, there are many species which produce peptone and a slightly bitter taste, there are some of them which produce an intensely bitter taste which is much more striking than that of the ordinary peptonizing organisms. There are, perhaps, two kinds of bitter milk, one caused, as Hueppe suggests, by peptones, and the other by some special product of a more intensely bitter nature than peptone. Further work is required to solve the problems here suggested.

ALKALINE CURDLING OF MILK AND THE PEPTONIZING POWER.

This is a third interesting subject associated with the alkaline fermentations. One of the common effects of the alkaline bacteria, though by no means a universal one, is the curdling of the milk. Now of course the alkaline reaction of the milk proves that the curdling can not be due to the formation of an acid as in the ordinary souring. Although Haubner [52] was the first to see that milk sometimes curdled without being rendered acid, it was Duclaux [82] who first tried to explain the matter. Duclaux found that certain species of bacteria certainly did curdle milk, though rendering them amphoteric or slightly alkaline in reaction. From many experiments he concluded that this curdling was due to some ferment or enzyme produced by the bacteria and having characteristics similar to those of rennet. He supposed that the bacteria produced this ferment in some way, probably as a secretion, and that the rennet thus formed curdled the milk. Duclaux further found that in these cases the curd was soon dissolved by the further action of the bacteria, the solution taking place slowly and seeming to be very similar to the solution in pancreatic digestion. The casein was certainly peptonized in a very similar manner. In his first papers Duclaux did not succeed in isolating the ferment nor obtaining

any positive evidence of its formation. In later papers [87] he described experiments which render it almost certain that such an enzyme is formed, although he did not isolate it in a pure state.

Hueppe [84] further verified the conclusion that curdling may take place in an alkaline medium and agreed with Duclaux that the only plausible explanation was the formation of a rennet-like ferment. He also studied the subsequent solution of the curd and concluded that it was not due to the same enzyme which curdled the milk, but to a second enzyme, which was very similar to trypsin. Thus the bacteria in question were supposed to produce two distinct soluble enzymes, one having rennet characters and the other tryptic characters.

Nothing more of importance was added to the question until the work of Warington [88], although Loeffler and others had verified the general conclusions of Duclaux. Warington studied several species of bacteria and divided them, as regards their action on milk, into three classes. One produced abundant acid and as a result curdled the milk. The second also produced acid but in small quantity—not sufficient acid indeed to curdle the milk of itself, as was shown by experiment, but the bacteria nevertheless did, in some way, curdle it. The third class curdled the milk, though rendering it alkaline. In the last two classes evidently some different action took place from that in the first, and Warington accepted the only suggestion that had been offered that the curdling was produced by a rennet-like ferment. The bacteria of both of the last two classes were found to have the further power of dissolving the curd slowly, and Warington noticed the important fact that all of the organisms which had the power of curdling the milk and subsequently dissolving the curd belonged to the class of bacteria which liquefy gelatin. He saw at once a close relation between these properties and predicted that all bacteria which liquefy gelatin would be found to possess the power of digesting milk casein. The prediction has been quite well substantiated by later work, and indeed is not surprising when we remember that the liquefaction of gelatin is really a digestion and therefore quite similar to the solution of the curdled casein.

At about the same time Duclaux [87] published a more thorough study of the action of these organisms. He conclusively demonstrated that the bacteria in question have two entirely independent actions on milk, due to two entirely different ferments. Although he was not able to actually isolate the enzymes, he did succeed in separating the two actions so as to get the one without the other. Cultivating the bacteria in milk for a few days he removed them by filtering the milk through porcelain, and thus obtained a filtrate which contained no bacteria but which was active both in curdling milk and in dissolving the curd. He found that the curdling would occur only at certain temperatures, while the digesting or gelatinizing power was manifest at almost any temperature. Hence, by using the proper temperature he could get the milk casein digested without first being curdled.

In more recent times these observations have been repeated many times by various observers, and the distinction of the two processes made even more clear. Weigmann and Conn have shown that there are some organisms which possess the digesting power without the curdling power. Under their influence the milk slowly becomes less white and more transparent and finally becomes entirely transparent, the result being the same as when the mass of curdled casein has become dissolved. But this occurs without any curdling, and the bacteria in question do not curdle the milk under any conditions. Conn has found that a certain species studied by him possessed at first both properties, but after long cultivation in gelatin it lost its curdling power completely, though retaining the digesting power intact. Finally Wood [89] determined that even the gelatinizing power may be lost by long cultivation in a bouillon containing carbolic acid in small amount. It is evident then that we are forced to the conclusion that certain species of bacteria give rise simultaneously to two distinct forms of fermentation in milk, one producing a rennet-like curdling and the other a tryptic-like digestion of the casein. So far as is known to-day the curdling power is always accompanied by a digesting power, but some instances are known in which bacteria have the digesting power without the curdling property.

The curdling of milk by these organisms is very similar to that produced by rennet, so similar indeed that it is usually regarded as identical. No special study of the curdling has been made to determine whether the chemical changes are identical with those of rennet. It is certain also that the bacteria readily curdle boiled milk, and this, as we have seen, is not true of rennet. It is possible, therefore, that the action is not identical with that of rennet. In regard to the chemical nature of the digestive process, we have very little information, and indeed the digestion does not appear to be at all uniform. One part of the action in all cases seems to be a peptonizing of the casein, and leucin and tyrosin are frequently formed. There is evidently a great resemblance to tryptic digestion. Indeed the similarity of the changes taking place in the digestive canal under the influence of rennet, pepsin, and trypsin, and those taking place under the influence of the growth of this class of bacteria, is very great. Duclaux has insisted upon their practical identity, and a very striking parallelism can be drawn between them. But when we follow into the details of the process we find a great variety among the effects of the different species of bacteria. The appearance of milk acted on by different species is very different. In all cases where a curd is formed it may be easily distinguished from that of the acid-forming class by being soft and slimy rather than hard and fragmented. After a few days it is seen to begin to be dissolved, but with very different results in different cases. Sometimes the curd becomes completely dissolved into a limpid liquid, while in other cases the solution is less complete, a considerable part of the curd remaining

in the milk undissolved, even after the bacteria have been growing for several weeks. The liquid solution which results is sometimes cloudy, sometimes clear, sometimes opaque, sometimes transparent; sometimes it is white and in other cases yellow, or amber colored, or red, or green; in short, there is the greatest variety in the solution resulting from the different species of bacteria [Conn, 90]. Of course all this tells us that different chemical changes are occurring in the milk, but beyond the few observations which have been made upon the superficial appearances, little or nothing has been done to tell us of the decompositions going on. The chemical side of alkaline fermentations is a wholly unexplored field, and doubtless much of importance remains for discovery in this direction.

As we have seen, the bacteria which produce these effects all belong to the class which liquefies gelatin, and Duclaux further states that the aërobic species alone have the power of giving rise to the rennet ferment, while the anaërobic species chiefly cause the milk to undergo putrefaction. The number of organisms which form these ferments is very great. Duclaux has studied a large number, to which he has given the generic name of *Tyrothrix*, and other bacteriologists have found the characters quite widely distributed. So far as the bacteria are concerned the object of the action seems to be for the purpose of rendering the milk suitable for their own food. The casein of the milk is not in condition to be absorbed by bacteria any more than it is to be absorbed by the digestive organs of man. The milk is therefore curdled and digested and the resulting solution is much in the same condition that it is after being acted upon in the human stomach. Actual analysis shows considerable increase in the soluble proteids, and peptones are always present. It would seem then that the bacteria as well as animals need to digest the casein before they can absorb it, and having no special digestive organs they produce digestive fluids, which they pour into the milk, thus digesting it outside of their body rather than inside, after the fashion of larger animals.

It has been stated that the actions produced by these organisms are due to the production of a chemical ferment or enzyme, but, as we have seen, this is taken partly for granted. In this question we are brought close to the theory of fermentation in general, but this has not yet been satisfactorily given. That fermentations are connected with the life of microorganisms is undisputed, but the theory that they are directly produced by chemical enzymes secreted by the organisms, while strongly advocated has not yet been either proved or disproved. Observations at the present time are certainly tending to show that in some cases at least bacteria act by the production of such enzymes. Several observers have in recent years succeeded in proving that solutions in which bacteria have been growing possess fermenting powers even when deprived of bacteria by filtration through porcelain, and in some instances soluble ferments have actually been isolated from such

solutions. There would seem to be very little doubt that the curdling and digesting of milk under the influence of bacteria are brought about by soluble chemical ferments. This is especially interesting when we remember that all attempts to get similar evidence of the formation of a ferment by the lactic organisms have failed. No one has yet found any reason for thinking that the lactic organisms produce a chemical ferment, and it would seem, in the light of present knowledge, that the acid and alkaline fermentations are entirely different in their nature, not only as concerns their results, but also in their method of action. Alkaline fermentations may be explained as due to chemical enzymes, but there is no evidence that acid fermentations can be accounted for in this way.

In the ordinary handling of milk the class of organisms included under the head of alkaline ferments is of little importance. They grow slowly and the lactic-acid-forming species usually get the start of them, producing their own marked effect on the milk, so that the action of the alkaline species is entirely obscured. Moreover the acid-forming species soon produced so much acid that the growth of all bacteria is checked and thus the alkaline species have no chance to produce much effect on the milk. At the same time these species are of the greatest importance in dairy matters. In the first place many of them form resisting spores which will endure high temperature and render it very difficult to sterilize milk by heat. The presence of this class of organisms in milk led experimenters astray for a long time, leading them to conclude that milk contained its own ferment since it could not be sterilized by ordinary methods of heating. Further, these organisms play an important part in dairy processes. They are always present in milk which has been standing for a short time, and sometimes their abundance is great enough to produce noticeable effects. Everyone who has had an extended experience with milk has seen instances of milk curdling without the usual acid taste, and it is a familiar fact that curdled milk is by no means constant in character. There is the greatest variety in the stiffness of the curd, the amount of the whey, the taste, odor, etc., and all of these differences are due to varying numbers and species of bacteria other than the lactic acid class. Among them the rennet class of bacteria is abundant, and their share in the ordinary fermentations of milk is not a small one, especially in cool weather. In the keeping property of butter they doubtless play a part, though they are not the sole cause of rancidity. In the ripening of cream for churning their part is still greater, and in the ripening of cheese they are of the utmost importance. Undoubtedly we may trace many of the difficulties of the butter and cheese maker to bacteria of this class. A further knowledge of their action will be of great value to the dairy interest. We are as yet only on the threshold of the study of these organisms, for while the lactic acid organisms have been quite carefully studied in past years the rennet-forming class have only recently come into notice.

BLUE MILK.

Historically, blue milk was the first of the various fermentations of milk which received any special study. Before it was conceived that souring was anything more than a normal property of milk it was claimed and practically demonstrated that blue milk was an infection. This fermentation, characterized by the deep blue color which has given its name, occurs sometimes as an isolated trouble in individual dairies and sometimes it has become so prevalent in certain localities as to be almost an epidemic. Various suggestions have been offered as to its cause, for blue milk has of course never been regarded as a normal process but has always been regarded as caused by some abnormal conditions. A favorite explanation has been to attribute the trouble to something which the cow had eaten or to some diseased condition of the cow, and these explanations were held long after it had been shown to be caused by microorganisms. It was attributed to an abnormal ferment first by Sternhoff in 1838.

The connection of blue milk with microorganisms was first made out by Fuchs [41], this being the first instance of the study of bacteria in milk. Fuchs found in blue milk certain organisms (*vibrios*), which were especially abundant in the spots where the milk was becoming blue. These organisms he sent to Ehrenberg, who was at that time making a study of bacteria, and Ehrenberg added them to his list of vibrios under the name of *Vibrio syncyanus* (subsequently called *Bacillus cyanogenus*). Fuchs further made quite a number of experiments with the organism, finding that it would produce the blue color in milk and that its growth could be prevented by the use of proper disinfectants. By the use of such disinfectants the blue color could be avoided. He found that the organism would not produce the blue color in all media, but that it was really the cause of blue milk his experiments left him no room to doubt. Blue milk he therefore regarded as a typical example of an infection and one that should be treated like other infections. The only means of preventing it, he said, was by keeping the organisms out of the milk, and this must be done by the exercise of great care and cleanliness in all matters connected with the dairy, and by heating very hot all vessels which had previously held any of the milk.

These conclusions of Fuchs sound very modern, and are, indeed, exactly in accordance with our present knowledge. It would seem that the microscopists were then just about ready to make the discovery of the important relation of bacteria to milk and its fermentations. But such was not the case even in regard to the infection of blue milk. The theory of fermentation advanced and so ably supported by Liebig soon drove all idea of microorganisms as a source of fermentation out of the minds of most scientists. For the next twenty years all the work done on blue milk led in a different direction. Gielen, Elten, Haubner, Mosler, and even Hoffmann, insisted that the explanation offered by

Fuchs was incorrect. The last two did indeed find organisms in blue milk, but did not regard them as special agents. Mosler [68] only found the common mold *Penicillium glaucum* present, and while thinking that this might have something to do with the blue fermentation, referred the chief effect to some condition of the cow. According to him, *Penicillium* under ordinary conditions produced the souring of milk, but under other conditions of the cow this same organism induced different changes in the milk and gave rise to the blue color. Thus for twenty-five years (1840 to 1865) blue milk was regarded as produced by some chemical ferment associated with the casein of the milk, and was always attributed to some condition of the cow. It is not worth our while to dwell upon the experiments of this period since they have all been replaced by others.

After Pasteur had given definite proof of the importance of bacteria in fermentations in general, microscopists began again to look for the cause of blue milk among the microorganisms. Erdmann [65] first reviewed the subject by discovering a bacterium in blue milk, which he said was similar to if not identical with *Vibrio butyrique* of Pasteur. This was of course a mistake, as were his further observations. The methods of bacteriological research were so crude at that time that he had no means of separating or distinguishing different species of bacteria, and he concluded that this organism was the cause of both blue milk and red milk. The pigment was in each case produced from the casein by the same organism, and whether it was blue or red depended upon conditions connected with the cow. The red pigment he regarded as fuchsin and the blue pigment he said was triphenylrosanilin.

After this work of Erdmann nothing was done upon blue milk for many years. Finally the matter was again taken up by Neelsen [80], who once more found the *Bacillus cyanogenus* of Fuchs, and again proved its causal connection with blue milk. The organism, he found, would certainly cause the blue fermentation to appear in any specimen of milk, but he noticed that the blue color did not appear until just as the milk began to turn acid. Upon other culture media he found that the blue color did not appear, neither sugar, gum, nor potatoes showing any trace of the blue under the influence of the organism. But while this work was valuable, Neelsen was led into error by not having pure cultures of the organism which he was studying, and his work led him to believe that the organism went through a long series of development changes, acquiring new forms and new characters under different conditions. The confusion thus arising was, however, soon eliminated by others. Neelsen sent his cultures to Koch, under whose direction it was found that Neelsen was working with a mixture of four different species of bacteria, only one of which was the blue milk organism. Later, Hueppe [84] also made a study of the purified cultures and found that the *Bacillus cyanogenus* when growing by itself possessed no power of turning milk blue. If inoculated into sterilized milk there is produced a grayish blue color. This color was, however, easily turned into

a brilliant blue by the addition of a little acid or by inoculating the milk with the lactic organism. It follows that the explanation of blue milk is a double one. Ordinary milk contains some of the lactic organisms, and these, acting in connection with the *Bacillus cyanogenus*, produce the brilliant blue which characterizes the infection.

Several later works upon blue milk have appeared. Fleischmann, Reiset, Heim, and Behe have all studied the organism and each added some details as to its characters, but the general facts above outlined remain unchanged. The organism has been supposed to act on the casein of the milk, for it has no effect on the milk sugar. But Gessard [91] has recently concluded that the blue pigment is really produced from lactic acid. It will produce the pigment when cultivated in solutions of casein and ammonium lactate or tartrate. Milk sugar is unaffected by it, remaining in undiminished quantity in the milk during the fermentation. When growing in ordinary milk the effect of this organism is very marked. For a few hours no change is noticed, but at just about the time that the milk begins to become acid some intense blue patches make their appearance. The appearance of the pigment in these patches seems to be due to the milk getting slightly coagulated from the effect of the lactic acid forming and the coagulum thus localizing the action of the blue milk organism. The faster the acid forms the quicker the coagulation appears and the smaller are the blue patches, while if the acid is produced more slowly the blue patches are larger and of a better color.

Where the blue milk organism comes from is unknown, nor have we any knowledge of the causes of the occasional epidemics of blue milk. There can be little doubt that the cause is always from some unknown source of filth. In some cases the trouble has been traced to a single cow in a large dairy and has been easily stopped by isolating the individual which is found to be the cause, or by carefully washing the cow's teats with a little weak acetic acid solution. Blue milk is always an infection due to outside contamination and its remedy is always to be found in care and cleanliness. It does not occur in the carefully kept dairy.

Blue milk appears to be harmless. It has been fed to animals, which eat it readily and without harm. Within a few years blue cheese has been brought to the attention of scientists, and has been attributed to the same organism which produces the trouble in milk. So far as known to-day blue milk is produced only by the single organism *Bacillus cyanogenus*. Gessard [91], however, has shown that there are several varieties of this organism, not all of which produce the blue pigment.

ALCOHOLIC FERMENTATION OF MILK.

Milk does not readily undergo the alcoholic fermentation. Theoretically, there seems to be no reason why milk sugar should not split up into alcohol and carbonic acid as well as into lactic acid, but experiment tells us that bodies which readily split up to form lactic acid do

not readily form alcohol. The strictly alcoholic fermentations are produced by yeasts rather than by a bacteria, but when yeasts are cultivated in milk it does not undergo the alcoholic fermentation, but commonly the normal lactic fermentation ensues. The lactic fermentation that occurs is probably due in all cases to some of the lactic bacteria which are accidentally added to the milk along with the yeasts, for there is no reason for believing that pure yeasts can give rise to a lactic fermentation.

Still it is well known that alcohol does sometimes appear in certain forms of milk fermentation. The question has, however, been long debated. Fourcroy, Vauquelin, Lagrange, Bacholtz, Vogel, and Hess discussed the matter from various standpoints in the first three decades of the century. The last two concluded that while milk sugar is not to be converted into alcohol, it is easily converted by various acids into galactose, and this was supposed to be subject to alcoholic fermentation. Lubolt [59] found small quantities of alcohol present in lactic fermentations of milk sugar. In his experiments the fermentation was produced by the addition of sour milk to solutions of milk sugar, and at the end of the fermentation it was found that a yeast had developed in the solutions. To this yeast he attributes the alcohol. The alcohol in this case was of course simply a by-product, and Lubolt's fermentation could not properly be called an alcoholic fermentation.

The formation of alcohol in the fermentations of milk sugar was confirmed by Hallier [67] and was later attributed by Musso to the fermentation of galactose produced by the action of lactic acid on the milk sugar. This explanation was certainly not correct, since later observers have proved that pure galactose will not ferment. But practically nothing was known in regard to the alcoholic fermentation of milk until bacteriologists turned their attention to two different beverages formed from milk which undoubtedly contain considerable quantities of alcohol. The first of these is koumiss. This beverage has been known from time immemorial by the nomadic tribes of Tartary, and is prepared by them from mares' milk. This milk readily undergoes an alcoholic fermentation. It is usually prepared by adding to the milk a little fermented koumiss or even a little ordinary sour milk. The milk does not sour typically, but it is converted in a couple of days into an alcoholic beverage. Within the last few years this drink has become somewhat common as a beverage for invalids, and is prepared from cows' milk. To prepare it a little cane sugar is added to the milk and then some common yeast planted in it. The fermentation that follows produces something practically identical with the koumiss from mares' milk. The casein is at first precipitated, but is subsequently in part dissolved. Its nature seems to be somewhat changed, for it is no longer coagulable by the ordinary methods and its dietetic value seems to depend upon the fact that the casein exists in a state of finely divided particles. This condition prevents the massive curdling which occurs in the stomach

when normal milk is acted on by the gastric juice, and its digestion would therefore seem to be more easily accomplished.

Quite a number of analyses of koumiss have been made, but it can not be said that they teach very much as yet in regard to the nature of the fermentation. Alcohol is a uniform product, and its quantity seems to increase daily for several days. The casein seems also to undergo great changes. Peptone, parapeptone, albumen, acid albumen, and hemialbumose have all been found in koumiss. The presence of these constituents would seem to indicate that the changes going on in fermenting koumiss are akin to those of ordinary digestion, or perhaps more correctly compared to the changes which occur in milk as the result of the growth of the peptonizing species of bacteria mentioned in an earlier section of this paper. The different analyses of koumiss do not agree very closely, a fact which is not at all surprising, for it is hardly probable that the fermentation will be identical in two cases. A variety of microorganisms must be present in all cases and the resulting fermentation must vary with conditions.

Very little is known, however, in regard to the relation of this fermentation to microorganisms. From the fact that in preparing koumiss from cows' milk it is necessary to add cane sugar before the alcoholic fermentation will begin, it seems probable that it is the fermentation of this cane sugar that gives rise to the alcohol, or at least begins the process. It is not known why mares' milk will undergo the fermentation without such previous addition of sugar, although analysis shows that there are some chemical differences between mares' milk and cows' milk. It is certain, however, that milk sugar can be made to undergo alcoholic fermentation, as we shall soon see, but we can not tell to what extent this process occurs in the formation of koumiss. It is safe to conclude that yeast has some share in the process, and also that part of the koumiss fermentation is due to the action of the peptonizing bacteria described in a previous section of this paper. Further, some of the lactic organisms are always present. By the combined agency of these bacteria and yeasts the milk is rendered acid, is provided with a certain amount of alcohol, and is partly digested. In this condition it is more easily handled by a weak stomach. Beyond these facts, however, our knowledge does not go at present.

The second alcoholic beverage has been more carefully studied, though it can hardly be said that we know much more in regard to it. It is the "kefir" of the Caucasus. It was first studied by Kern [82], who discovered that the people of the Caucasus had long been accustomed to manufacture an alcoholic drink from cows' milk. This drink had been known to science as early as 1784, but no special attention had been paid to it. As soon as Kern had brought it into notice a number of chemists and microscopists began to study it, and a large number of publications appeared in the next few years, prominent among which those of Kranhals [84] and Streuve [84] may be mentioned.

Kefir is usually made in leather flasks. The fermentation is started by placing in the milk some small bodies called "kefir grains." These grains contain the fermenting organisms, and they not only start the fermentation but they appear to grow somewhat in size during the process. After the kefir is prepared the grains are taken out again, dried, and kept for future use. When dried, these grains will keep for years with their power undiminished. Of course no one knows where the original grains came from, since they have been handed down from generation to generation, their origin being lost in the past. When these grains were first studied, they were said by Kern and Kranhals to contain three separate organisms. One was a yeast, in all respects like the ordinary yeast; the second was an ordinary bacillus; while the third was also a bacillus, but of a peculiar character and thought to be the characteristic organism of kefir. This bacillus was described as forming a large spore at either end, which gave to it the appearance of a dumb-bell, and it was named *Dispora caucasia*. Kranhals also found that the grains sometimes become sour or slimy from the presence of an unusual quantity of bacteria in them. Streuve [84] also found both bacteria and yeasts in the kefir grains, but he found other organisms also. *Oidium lactis* was common, and he found chains of *Leptothrix* also present. Streuve, however, denied the existence of the *Dispora caucasia*. He found bodies which certainly resembled the organism described under this name, but he concluded that they were not organisms but simply fibrillæ of leather derived from the flasks in which the kefir had been made. The kefir grains were again studied by Nencki and Fabian [87], and they found a still larger number of organisms. They identified *Saccharomyces cerivisiae*, *Bacillus acidi lactici*, *Oidium lactis*, and *Bacillus butyricus*, but they also were unable to find the *Dispora*. Later Adametz [90] found still other organisms. He found those already mentioned, with the exception of the *Dispora*, and also several species of bacteria which belong to the class capable of curdling milk and then digesting the curd. He found three species of yeasts, none of which seemed to be the common *Ceravisiae*, and he found a single species of mold always present. From all this it will follow that the kefir fermentation can not be a simple thing, but one in which a large number of organisms take a part.

As to the relation of these organisms to the process and the chemical nature of the fermentation, various opinions have been held from the start. It was thought by Streuve that the yeasts alone were concerned in the manufacture of kefir. But this is certainly not true, for it was later shown by Duclaux that the yeast found in the grains would not alone produce any alcoholic fermentation. According to Beyerinck, however, one of the kefir yeasts has this power. But evidently the process can not be a simple alcoholic fermentation of milk sugar. The first suggestion offered to replace that of Streuve was that the lactic organism, which seemed to be always present, produced at the outset

the typical alcoholic fermentation of the milk sugar, and at the same time partly effected its hydrolysis. The yeasts present were then supposed to act upon the products of the inversion, which they were able to convert into alcohol, while the third organism (*Dispora caucasica*) effected a partial gelatinization of the casein. All of this seemed to be in harmony with the fact that during the first twenty-four hours of the kefir fermentation there was a rapid increase in the amount of lactic acid, while during the next day there was a larger production of alcohol. Later theories suggested that the *Dispora* was a special agent to invert milk sugar and thus render it fermentable by yeasts. Musso had earlier suggested that the lactic acid might convert milk sugar into galactose, which is fermentable, while Cochin thought that a diastase was produced in the milk which rendered the milk sugar fermentable.

More recent study has made considerable additions to our knowledge, though it has by no means satisfactorily solved the problem. The *Dispora caucasica*, as we have seen, is no longer regarded as an organism, but we have found that the kefir grains contain a considerable number of organisms instead of three, and this fact, together with our advancing knowledge of the variety of fermentations produced by different organisms, has taught us that the kefir fermentation must be a very complex affair. To begin with, it has been found that there are some species of yeasts that are capable of producing an alcoholic fermentation of milk sugar unassisted by other agencies. This appears to be true of one of the species of yeast that is found in the kefir grains (Beyerinck). It may be true, therefore, that the alcoholic fermentation is due to this organism alone, but certainly the alcoholic fermentation is accompanied by other actions produced by the other organisms present. This is shown by the chemical analyses which indicate a complicated set of decompositions. Quite a large number of analyses have been made which show the presence of casein, lactalbumen, fats, salts, lactic acid, alcohol, peptone, glycerin, succinic acid, butyric acid, acetic acid, syntonin, and various other compounds in less amount. Milk sugar is always found, thus showing that the fermentation of this product is never complete. The analyses of different chemists do not agree, and, indeed, it is found that the composition of kefir changes from day to day, some of the products increasing and others decreasing as the fermentation advances. All of this is exactly what we should expect when we remember the number of kinds of organisms present in the kefir grains. We must indeed look upon the kefir fermentation as due to a complicated set of decomposition changes produced by a variety of organisms, some of them giving rise to alcohol from the milk sugar, some coagulating the casein, some producing peptone by the process of digestion above mentioned, and others producing a variety of acids and other bodies which will vary more or less in different cases. What constitutes a typical kefir fermentation we can not tell. We do not even

know how far these various secondary decompositions are necessary for the formation of the beverage. It is certain that as ordinarily made kefir is the result of a very complicated set of fermentations, one of which results in the production of alcohol.

There is evidently a considerable similarity between kefir and koumiss, since each is essentially an alcoholic fermentation of milk sugar accompanied by secondary changes. Chemical analyses also tell us of many similar secondary products, so that the two beverages are quite alike, but at the same time there are some points of difference. In the first place, one is made directly from cows' milk, while the other can be made from cows' milk only after the addition of a little cane sugar. Moreover, the chemical analyses seem to indicate that the different constituents are present in different proportions. But until a more careful study is made of each of these processes, it is impossible to state how far they are alike and how far they are due to different fermentations. That a different set of organisms is concerned in the manufacture of koumiss and kefir is certain, and we may be justified, therefore, in regarding the details of the fermentation as different in the two beverages. The dietetic value of each is due not to the alcohol produced, but chiefly to the peptonized condition of the casein, and this, as we have seen, is due to the action of the class of peptonizing organisms. In essential features, therefore, koumiss and kefir are alike.

Koumiss and kefir are thus alcoholic fermentations of milk accompanied by various other fermentations. Within recent years, however, several bacteriologists have found that there are a number of organisms capable of producing an alcoholic fermentation of milk sugar when acting alone. Piroitta and Robini [79] described an alcoholic fermentation of milk sugar due to a special yeast (*Saccharomyces galacticola*), but it is doubtful whether they had pure cultures. Duclaux [87] was the first to study with modern methods the relation of pure cultures of yeast to milk. From kefir grains he isolated a species of yeast which was not capable of producing an alcoholic fermentation of milk sugar, and from this he was led to the study of several other species of yeasts, all of which gave similar negative results. In all of his experiments there seemed to be a small amount of alcohol produced, but the chief effect of each yeast was to oxidize the sugar, and there was no proper alcoholic fermentation. Subsequently he obtained a specimen of milk which had spontaneously undergone an alcoholic fermentation, and from this milk isolated a species of yeast which differed from the others in various morphological points, and also in being distinctly aerobic. This yeast did not oxidize the milk sugar, but produced an abundant alcoholic fermentation of the milk. During its growth all of the milk sugar was converted into alcohol, none remaining in solution after the fermentation was completed. This would indicate that this is not the cause of koumiss and kefir fermentation, since, as we have seen, there is always some milk sugar left in these beverages even after the most complete fermentation. The yeast

of Duclaux does not really coagulate milk, but it renders it slightly viscous, so that it will readily coagulate with heat. Duclaux tried in vain to isolate some form of chemical ferment from it, but was forced to conclude that none was produced and that the yeast acted through its growth directly and not by the formation of an enzyme. In general he concluded that milk sugar is a more stable product than cane sugar, and that most yeasts are consequently unable to effect its decomposition. This yeast, being an aërobic organism, was more powerful and could readily act upon the sugar.

Since the work of Duclaux, others have succeeded in finding yeasts which produce an alcoholic fermentation of milk sugar. Adametz [89] two years later described a yeast with a similar power, and which he thought was a distinct species from the one found by Duclaux. He named it *Saccharomyces lactis*. Duclaux, however, thought that it was the same species as found by himself, though perhaps differing slightly, and Kaiser has more recently found a form intermediate between the one described by Duclaux and that of Adametz. Grotenfelt [89] found that alcohol was produced by certain lactic organisms. Meantime Martinaud [89] had been experimenting upon the fermenting power of common yeasts, and has found that most species of common yeast would produce an alcoholic fermentation of milk provided a small amount of cane sugar is previously added. The amount of cane sugar necessary to add was different with the different species of yeast, and the amount of alcohol produced was also variable. In these experiments the milk was commonly found to coagulate, but not by the formation of an acid, for Martinaud found that the filtrate obtained by passing such milk through a porcelain filter had no power of curdling milk. From this he concluded that the fermentation and coagulation were physiological processes connected with the growth of the yeast cells and not associated with the formation of any chemical ferment.

The last work on the subject is that of Beyerinck [89], which in a measure contradicts previous observations. He studied two species of yeast, both of which were capable of producing alcohol from milk. One of them he isolated from kefir grains and the other from Edam cheese. Beyerinck proved that these yeasts *did* give rise to a chemical ferment during their growth. The enzyme thus produced has the power of inverting milk sugar, and he named it *lactase*. Lactase is produced by both of the yeasts which ferment milk, but he found that it is not produced by the yeasts which do not have the milk-fermenting power. Since it is only yeasts which produce lactase that have the power of producing alcoholic fermentation of milk, he concluded that yeasts must first invert the milk sugar before they can ferment it.

It will be seen, then, that while the alcoholic fermentation of milk is not very common, it is nevertheless a well-defined type of milk fermentation. There are at least two or three species of yeast which can produce it, perhaps by producing first an enzyme which inverts the

milk sugar. There are two well-known beverages which are produced by a complicated fermentation of milk, the formation of alcohol being the most prominent feature. It appears that small amounts of alcohol are produced in some of the common forms of lactic fermentation, and finally, we have found that almost any species of yeast can produce alcohol from milk if the process be first started by adding to the milk a little of the easily fermentable cane sugar. So well known is this alcoholic fermentation to-day that it has recently been suggested that the use of whey in the manufacture of alcohol on a large scale would be a profitable undertaking.

SLIMY FERMENTATION.

A slimy fermentation of milk is a somewhat common occurrence and occasionally produces great trouble in dairies. In Norway and Sweden the people are fond of slimy milk as an article of diet and have found a method of producing it artificially. This is done by rubbing the interior of milk vessels with a plant by the name of *Pinguicula vulgaris*, or sometimes by immersing the stem of this plant in the milk, or even by feeding the plant to the cows. Slimy milk has an important bearing upon the manufacture of Edam cheese. But elsewhere this fermentation is a troublesome one, since it destroys the milk for all ordinary uses. Such milk will furnish no cream. It can not be churned and is ruined for drinking purposes.

There have been the greatest variety of theories as to the cause of slimy milk. Diseases of the mammary gland, variations in the food of the cow, and differences in conditions surrounding the dairy have all come in for a share in the explanation. But slimy milk, with the majority of other fermentations, has finally been traced to the growth of microscopic organisms which get into the milk subsequent to the milking.

Slimy fermentations of various media have been known since the beginning of this century, and many chemists studied the subject before 1860. But the work that was done has now very little value, since it was instigated by wrong ideas and erroneous conceptions. It was Pasteur who first turned our thoughts in the right direction by discovering that there was a special "yeast" which had the power of giving rise to a slimy fermentation of milk sugar, producing therefrom a sort of gum (mannit) and carbonic acid. These were constant products of the fermentation, while lactic acid, butyric acid, and hydrogen gas were frequent by-products. Pasteur was soon able to make practical use of this discovery in suggesting a remedy for the prevention of certain diseases of wine which had caused great trouble to vintners. "Ropy" wine is indeed really a slimy fermentation.

The first mention of a slimy fermentation of milk was by Lister [73]. Among other fermentations (all of which he attributed to the same organism) Lister found one which rendered milk ropy. So viscous did

the milk become that after forty-eight hours the vessel in which it was held could be inverted without the milk spilling. Soropy was it that it could be drawn out into fine, silky threads. Lister attributed the trouble to the growth of bacteria, although confounding the organism with the one which produced the ordinary souring of milk. In a later paper [77] he corrected this mistake, although he did not discover the special organism which produced the slimy fermentation.

Our first systematic information on slimy milk was derived from Schmidt-Mühlheim [82]. This observer isolated from a lot of slimy milk a definite organism which he conclusively proved to be its cause. The organism was certainly different from any which Lister described, but from the impurity of Lister's cultures it may be that this organism was the same one as that which he had. The organism described by Schmidt was a micrococcus, frequently united into chains, and was seemingly identical with one found the year before by Bechamp [81] in certain slimy fermentations and named by him *Micrococcus viscosus*. The organism grows best at temperatures between 30° and 40° C., but is killed by a temperature of 60°, although when dried it may be heated to 100° without injury. Its action was on the milk sugar and not on the casein, for it was found that solutions of milk sugar were rendered slimy by the organism, provided enough peptone was added to the solution to make a proper nutrient solution for the organism. Solutions of cane sugar, grape sugar, or mannit were found to be similarly affected. On the other hand it would not produce a sliminess in either casein or albumen. The slimy substance that was formed was precipitated by alcohol as a white, sticky mass, slightly soluble in water and colored brown by iodide of potassium. No mannit appeared to be formed in this fermentation, as had been in the fermentation described by Pasteur, nor was carbonic acid evolved. Lactic acid and butyric acid were produced and the milk was consequently acid in reaction. The slimy mass contained no fat, that compound seeming to have passed from a condition of emulsion to one of solution. But although these several facts were determined, very little was known in regard to the chemistry of the fermentation. It was evident that the organism of Schmidt was a different one from that found by Pasteur.

These observations of Schmidt were independently confirmed in part by Eugling [82]. His studies concerned the chemistry of the fermentation chiefly, but incidentally he noticed bacteria associated with the milk and attributed the trouble to them. He found that the trouble was infectious and could be prevented by various disinfectants as well as by boiling the milk. To avoid the trouble he simply recommended the most strict cleanliness in regard to all matters pertaining to the dairy.

From this time a slimy fermentation was recognized as a common infection of milk and numerous miscellaneous observations began to accumulate. It soon appeared that this fermentation was also produced

by a large variety of organisms instead of a single species. Hueppe [84] mentioned a micrococcus with this power, but gave no description of it. Flügge [86] mentions the slimy fermentation as produced by *Bacillus mesentericus*. Van Tieghem found that *Leuconostoc mesenteroides* produces the viscous condition. Duclaux [87] described a bacillus of slimy milk to which he gave the name *Actinobacter du lait visqueux*. In the same work he described a second species, *Actinobacter polymorphus*, which at first renders the milk slimy, but subsequently dissolved it into a liquid whose viscosity is about the same as that of normal milk. Loeffler [87] isolated an organism which rendered milk slimy and thread-forming (*i. e.* capable of being drawn into threads). It was a bacillus forming short segmented threads and producing a strong acid reaction in milk and a peculiar odor. Whether it acted on the milk sugar he did not determine. Hess and Borgeaud [88] found a streptococcus with a similar power. Weigmann [89] isolated from the whey of Edam cheese a coccus which showed a tendency to form chains of four to ten cells. This organism rendered milk slimy in ten to fifteen hours, at an optimum temperature of 30° to 40° C. Later a small amount of lactic acid was formed and the milk curdled in small flakes. Weigmann also found this same organism in the artificial slime produced by the use of the plant *Pinguicula*, as above mentioned. Van Laer [89] isolated two bacilli from the wort of beer, which possessed the slimy fermenting power. They could render milk viscous, although they have not been found in milk which has become slimy from natural sources. Freudenreich [90] and Gillebau have studied a bacillus obtained from cheese, which renders milk remarkably slimy at 18° C. At 37° the milk was curdled rather than rendered viscous, and the effect was not so marked in sterilized milk as in normal milk. Adametz [89] found and very carefully studied an organism (*Bacillus viscosus*) which occurs in brooks and seems to be quite widely distributed. It acts slowly, requiring four weeks to complete its effect. As in the case of Schmidt's organism, the fat is changed from a condition of emulsion to one of solution. Nocard and Mollereau [89] isolated a streptococcus from inflamed udders, which rendered milk slimy and seemed to be identical with the organism of Hess and Borgeaud. Schultze isolated and Ratz [90] carefully studied an organism which renders milk slimy after eight days. The milk is curdled and rendered acid. Freudenreich finds other slime-producing organisms in diseased cheese. Storch [90] mentions two bacteria capable of rendering milk sugar slimy without the presence of casein. Finally, Conn has described a micrococcus which belongs to the rennet ferment-producing class, curdling the milk and subsequently dissolving the curd. The solution that results is marvelously slimy. This organism produces an intensely bitter taste and was found to be present in certain specimens of bitter milk in the dairy. In addition to the above, several organisms have been found which render certain solutions slimy, but do not have this effect on milk.

From all of this it will appear that the slimy fermentation of milk is connected with a large variety of organisms. It must not be understood, however, that the fermentation is the same in all of these cases; on the contrary, there is no doubt that there are several different classes among these organisms. Even in their general effect on milk there is a wide difference. Some of them give to the milk only a slight sliminess, while others render it tenacious almost beyond belief. The organisms described by Ratz and Van Laer, for instance, only produce a moderate viscosity in milk, while that described by Conn renders the milk and other solutions so slimy that it can be drawn into threads 10 feet long but so small as to be hardly visible. Indeed, slimy milk and ropy milk seem to be distinct forms of fermentation, but we can not yet draw any line between them. Some of the organisms render milk slimy in their early growth, others only after several days, and some do not render the fresh milk slimy at all, but first curdle it and then digest the curd into a slimy solution.

So far as their chemical side is concerned, the fermentations are also widely different from each other, although not sufficient is known to enable us to classify them all at present. The slimy substance is a different chemical body in different cases, and is derived from different sources. Sometimes it appears to be a secretion from the bacteria themselves, while in other cases it appears to be produced as a decomposition product. In some cases the slimy substance appears to be related to cellulose in its chemical nature, in others it is a special form of albuminoid, while in still other cases it appears to be a nitrogen-holding compound but not an albuminoid. Mannit, carbonic acid, lactic acid, butyric acid, peptone, etc., are produced by the various organisms, and some of them will ferment glucose while others will not. In regard to several of them we have as yet no knowledge of their chemical action.

From the list given above it will be seen that some eighteen distinct organisms have been described as associated with the slimy fermentation, all of which have the power of rendering milk slimy. Not all of these have been found in milk, however. About half of them have been found occurring spontaneously in milk and may therefore be regarded as possible sources of the normal slimy fermentation of dairy milk; the others being found elsewhere can only be regarded as remote possibilities in connection with the milk problem. But of those normally occurring in milk, perhaps six (the organisms of Schmidt, Weigmann, Hueppe, Duclaux, Eugling, and Freudenreich) have been found under conditions which warrant us in supposing that they may be the cause of the troublesome infections of slimy milk which occur in the dairy. Such spontaneous slimy fermentation has not yet been studied in a sufficient number of cases to enable us to tell whether it is usually caused by the same organism or by a large number of species, as we have seen to be the case with the lactic fermentation.

In the history of slimy milk various suggestions have been offered as to its cause. Perhaps the most favored one has been to attribute the

trouble to something that the cows have eaten. The fact that the people of Norway can produce slimy milk by feeding their cows with the plant *Pinguicula* seems to lend force to this idea. But there is no question, in the light of our present knowledge, that uncontaminated milk will not undergo either the slimy fermentation or any other, no matter what the cow has eaten, provided the cow be healthy. The whole secret of the trouble must be found in the contamination of the milk after the milking, either from the milk vessels or the hands of the milker, or perhaps from germs in the air. It is easy to see how the milker in handling the above-mentioned plant might infect the milk in some accidental manner. It is easy to understand that various organisms may be concealed in the hay or other food of the cow, and that the stirring up of these foods would fill the air with germs which could then get into the milk during the milking process. The fermentation that they produced would naturally be attributed to the cow's food. It would indeed be due to the food, but not to the food that the cow had eaten, but rather to that which the milker had handled. Cleanliness in the barn and dairy is a sure cure for the slimy fermentation of milk.

MISCELLANEOUS FORMS OF FERMENTATION.

In addition to the above well-marked classes of fermentation there are various others not so well known or so carefully studied. The numerous forms of bacteria and yeasts that grow in milk produce a large number of forms of decomposition of the milk sugar and the proteid factors. As a rule the yeasts affect the milk sugar while the bacteria affect either the sugar or the proteid elements. At present very little can be said in regard to the miscellaneous types of decomposition.

Among them are some organisms that produce especially striking effects from the production of pigments. *Blue* milk has already been noticed, but several other pigment-forming species of bacteria are known. *Bacillus violaceus* turns milk a violet color, and the same is true of the closely related *Bacillus janthinus*. *Yellow* milk has been known much longer, being indeed described by Ehrenberg and Fuchs as long ago as 1841. Schröter studied it later and attributed it to the same organism found by Ehrenberg, *Bacillus synxanthus*. List described several organisms from the feces of the sheep, some of which had the power of turning milk yellow. Adametz [90] isolated another species from ripening cheese. Conn [90] has found several more. These various organisms of yellow milk do not act alike. Some of them produce quickly a brilliant yellow milk; some of them belong to the rennet-forming class, first curdling the milk and then dissolving the curd into a yellow liquid. In these cases the resulting liquid is sometimes a brilliant lemon yellow, at other times it is orange-colored, and at other times it is amber-colored. Several species of bacteria turn the milk *green*. These also are bacteria of the peptonizing class and the green color appears only after the casein is peptonized.

Of the various pigments formed by bacteria in milk, perhaps the *red* pigment has been studied most. Quite a number of organisms are now known to produce this effect. The well-known cause of bloody bread, *Micrococcus prodigiosus*, will produce red spots in milk, especially in the cream at the surface, but it does not turn the milk so generally as to produce what would be called red milk. Erdmann [65] first studied a proper red milk and concluded that it was produced by the same organism that causes blue milk. This was of course an error. Schrodt [88] described and studied chemically some red milk, but did not discover its cause. Hueppe, in 1886, was the first to isolate a distinct bacterium capable of producing red milk and named it *Bacillus lactis erythrogenes*. This organism was subsequently more carefully studied by Grotenfelt [89]. It is an organism which has not the power of curdling milk and it produces the red color only in an alkaline medium. The color develops best if the culture is kept in the dark. Baginsky isolated a species from feces which turns milk red. It is similar to Grotenfelt's organism and is probably identical with it. Lustig [90] isolated a species from river water which curdles milk, but turns it red in about forty hours. Menge has described a *Sarcina* with a similar power, curdling the milk and subsequently dissolving it into a brownish red liquid. Demme [89] has described a yeast which renders milk red without coagulating it. It is evident from the various observations that the formation of a red pigment must be regarded as a property of quite a large number of organisms, but beyond these facts very little is known about it.

Leaving the organisms that produce a pigment in milk and turning to the other forms of miscellaneous fermentation, we find it impossible to proceed. The classes mentioned thus far have certainly not exhausted the types of fermentative decomposition which take place under the influence of bacteria, but of the others we do not yet know enough even to arrange them into classes. We have seen in our review that the conception of the fermentations of milk has been constantly widening. At the beginning of this century only one form of fermentation was recognized, but this was resolved into two about the year 1840, rennet curdling and the souring of milk being then first separated. At about the same time blue milk and yellow milk were recognized as distinct infections, but beyond this all of the spontaneous changes which occurred in milk were regarded as identical, or at least were not clearly distinguished. After miscellaneous studies, which proved nothing definite, Pasteur at last succeeded in resolving the spontaneous fermentations of milk into two forms, one giving rise to lactic and the other to butyric acid. Lister was the next to extend the subject by showing that there were several distinct forms of spontaneous fermentation which could be produced by allowing milk to stand in different localities. He first recognized slimy milk as a distinct type of fermentation. Following Lister's work a number of types of fermentation were recognized, but

there was no great advance until the invention of the Koch method of isolating pure cultures of bacteria. After this method came into use there was a rapid differentiation of the types of fermentation already recognized. Each type which had hitherto been regarded as produced by a single species of bacterium was now found to be produced by a large number of species, and the old forms of fermentation were retained only as class distinction. In addition to this, new types of fermentation were discovered, the discovery of the production of a bacteria rennet, of a tryptic ferment, of red milk, etc., all following each other rapidly. But each year bacteriologists are recognizing the necessity of a still wider differentiation. Each of the classes already described is found to contain several subordinate types of more or less distinctness. The species under each of the classes recognized all show the character of the class, but differ in other respects; for instance, all of the alkaline organisms agree in rendering the reaction of milk alkaline, but some of them curdle it while others do not; some of them produce digestive ferments while others do not; and when we go more into details we find even greater differences. Hardly any two of them agree in the odor, the flavor, or the color which they produce in the milk, and various other differences plainly prove to us that each has its own peculiar effect upon the milk. But of these secondary effects we do not know enough to-day even to attempt a classification. There is no doubt that these processes are of great significance in dairy matters, especially in the ripening of cream and cheese, but beyond recognizing their existence in great variety we can do nothing at the present time.

The fact is that milk is an excellent medium for bacteria growth. It furnishes proper food for all of the saprophytic bacteria and the various organisms of the air or the water may grow in it to almost any extent. The study of its fermentative changes resolves itself, therefore, into the study of fermentation in general. Fermentation, decomposition, putrefaction, etc., will all run into each other in the study of the changes occurring in milk, and it is impossible to draw any line separating them. For a complete knowledge of the fermentations of milk we must wait until we understand thoroughly the process of fermentation and decomposition in general. At present this is an almost unknown field. We can pick out a few of the simpler, more striking types of fermentation and group them into classes as we have done, but we must leave for future study the miscellaneous forms of decomposition and fermentation whose existence we recognize, but of whose nature we are entirely ignorant.

PRACTICAL BEARINGS OF THE SUBJECT UPON DAIRYING.

After this historical review of the fermentations of milk, the question of their practical bearing forces itself upon our attention, and this paper would not be complete without a brief reference to this subject. While many of the facts above outlined belong to pure science, it is

becoming more and more evident every year that their bearing upon dairying is of the utmost importance. The practical application of our knowledge of the fermentations of milk will concern each of the three chief dairy products, milk, butter, and cheese.

HANDLING OF MILK.

To those dealing with milk itself in any form, the various fermentations are especially undesirable and are constant sources of trouble. Such persons want the milk pure and sweet, and any of the various forms of fermentation injure the milk for their purposes. Now, so far as these matters are concerned, our study of milk fermentation has taught us first of all *that all fermentations of milk, even the common souring, are due to the contamination of the milk with something from the exterior after it is drawn from the cow.* If they are thus all due to contamination from without, all that is needed to prevent them is to treat the milk in such a way that no such contamination is permitted. But simple as this is in theory our study has shown us that it is a matter of practical impossibility. The lactic organisms of various kinds are so numerous and so common everywhere that no practical method can be devised for keeping them out of the milk. The person who handles milk must therefore recognize their presence in the milk as inevitable, and he must simply turn his attention to means of reducing them to the smallest number and keeping their growth within the smallest possible compass. This has been shown to be accomplished best by two precautions, absolute cleanliness and low temperatures. The great source of these organisms is in the unclean vessels in which the milk is drawn and in the filth which surrounds the cow. By scrupulous cleanliness in the barn and dairy the number of organisms which get into the milk may be kept comparatively small. The statement of a dairyman that "one should make as careful a toilet for the milking yard as for the supper table" is no exaggeration. Of equal value in preserving milk, is the use of low temperature, and to be of the most use it should be applied *immediately* after the milk is drawn. When drawn from the cow, milk is at a high temperature, and indeed at just the temperature at which most bacteria will grow the most rapidly. Under the influence of the atmospheric temperature, especially in the summer, the milk will become cool very slowly, but never become cooler than the air. The bacteria which have gotten into the milk will therefore have the very best opportunity for rapid multiplication and the milk will sour very rapidly. If, however, the milk is cooled to a low temperature immediately after it is drawn, the bacteria growth is checked at once and will not begin again with much rapidity until the milk has become warmed once more. This warming will take place slowly, and therefore the cooled milk will remain sweet many hours longer than that which is not cooled. It frequently happens from this cause that a milkman finds that his morning milk will sour earlier than the milk of the

night before. The milk drawn in the evening is put in a cool place at once and becomes quite cool during the night, while the morning's milk is at once put in cans and taken for delivery. It will thus happen that the older milk will actually keep longer than the newer milk, simply because it has been cooled and must be warmed before bacteria can begin to grow very rapidly. A practical knowledge of this fact will be of great value to every person handling milk. Early cooling to as low a temperature as is practicable is the best remedy for too rapid souring of milk.

A second lesson of no less importance has been taught. All of the *abnormal* fermentations of milk, such as blue milk, red milk, slimy milk, tainted milk, etc., are also due to the growth of organisms in the milk, and *all* of these are *preventable by care*. While the lactic organisms are so common and so abundant as to make it hopeless to try to keep them out of the milk, this is not true of the organisms producing the abnormal fermentations. These organisms are not so abundant, and by the exercise of care they may all be prevented from getting into milk in such a way as to cause trouble. If a dairy is suddenly troubled with slimy milk or any other abnormal trouble, the dairyman may feel sure that the cause is to be found in some unusual contamination of his milk and that the remedy must be extra cleanliness. He need not look for the cause in the food that the cow has eaten, but may perhaps find it in the hay which the milker has handled or in the dust which has been stirred up in the milking shed. He must look for the trouble in something apart from the cow, and usually in his own carelessness, either in the barn or the dairy. Search in this direction will usually enable him to remove the trouble, while experiments upon the food or conditions of the cow will usually be worthless. Sometimes such troubles may be traced to an individual cow among a large herd. This will of course indicate that this cow has in some way become contaminated with a source of filth which produces the trouble. We must always remember that with the healthy cow all contamination of the milk must come from the outside, and the remedy is seen at once. Such a cow should be cleaned, and especial care should be taken to carefully wash her teats with a weak solution of acetic acid for the purpose of removing whatever bacteria may be clinging to them. Such methods will soon remove the trouble. The second lesson for the dairyman is, therefore, that *all abnormal fermentations may be prevented by sufficient care and cleanliness*.

It is well to notice that certain abnormal odors and tastes in milk may be produced directly by the food eaten by the cow. If a cow eats garlic or turnip the flavor of the milk is directly affected. Various other foods may in a similar manner affect the taste of milk, but this class of taints may be readily distinguished from those due to bacteria growth. The odors and taints due to the direct influence of the food are at their maximum as soon as the milk is drawn, never increasing

afterwards. But the taints due to bacteria growth do not appear at all in the fresh milk, beginning to be noticeable only after the bacteria have had a chance to grow. If, therefore, a dairyman has trouble in his milk, which appears immediately after the milking, he may look for the cause in something that the cow has eaten; but if the trouble appears after a few hours and then grows rapidly worse until it reaches a maximum, he may be assured that the cause is some form of fermentation, and that the remedy is to be sought not in changing the food of the cow, but in greater care in the management of the dairy or barn.

Various methods have been devised for destroying the organisms after they have got into the milk. Numerous chemicals have been used, and several methods of using heat have been devised. Into the details of this subject we can not go at present. The methods have been devised for the consumer of the milk rather than the dairyman, and the latter need not concern himself with them. The lessons for the dairyman to learn from the study of the fermentations of milk, are scrupulous cleanliness in all affairs relating to milk, care in the dairy, thorough washing with boiling water of all milk vessels, and low temperatures applied to the milk immediately after it is drawn from the cow.

BUTTER MAKING.

To the butter maker the bacteria of milk present a different aspect. To him they prove friends instead of enemies. After the cream is separated from the milk it proves of advantage to the butter maker to allow bacteria to grow in it before churning. It is the custom of butter makers to allow their cream to "sour" or "ripen" for a number of hours before churning. This is accomplished by allowing it to stand in a warm place for twelve to twenty-four hours. During this time the bacteria in it are multiplying rapidly and of course producing the initiatory steps of the various forms of fermentation of which they are the cause. Prominent among them will be some of the lactic organisms, and these will produce the souring of the cream. But the changes which occur are not confined to the lactic organisms, for the warm temperature will hasten the growth of various other organisms which happen to be present in the cream.

The butter maker finds certain advantages in such ripening. He finds that the cream will churn more easily and that he can get a larger amount of butter from a given amount of cream if it is ripened than he could if it were churned while fresh. He finds further—and this is perhaps the chief value of ripening cream—that the butter made from ripened cream has a superior flavor to that made from sweet cream. To obtain a proper flavor or aroma is one of the chief objects of the butter maker.

Taking up the last matter first, we notice that the aroma is undoubtedly connected with the decomposition products of the bacteria growth. The volatile acids supposed to give flavor to the butter are not present

in fresh milk, but only appear after standing, *i. e.* after the fermentations have begun. For a time it was thought that the aroma of butter was due to some alcohol-like product formed during the ripening, or to the presence of lactic acid itself. In accordance with this last idea lactic acid has been used artificially to ripen cream, but without much success. Of course after we have learned that microorganisms have been forced to grow in the cream during the ripening, and when we combine this with the facts which we have learned of the fermentation products of microorganisms, we are forced to believe that the ripening of cream is a more complicated process than the simple production of lactic acid. The first person to investigate this matter, in the light of modern discoveries, was Storeh [90], a Swedish scientist. He assumed that the butter aroma was due to the growth of organisms and made a study of the bacteria in butter and cream for the purpose of finding, if possible, the proper species of organism for producing the aroma. After considerable search he finally succeeded in isolating from ripening cream a single bacillus, which seemed to produce the proper butter aroma when it was used in pure culture to ripen cream. Shortly after this Weigmann [89 and 90] studied the same phenomenon and reached similar results, also succeeding in obtaining cultures of an organism which produced a normal ripening and gave rise to a proper aroma. This ferment is coming into use in some of the creameries in Germany, the claim being made for it that it insures certainty in the result of the ripening process. It has not yet been introduced into this country as a practical ferment.

The value of using such a ferment, if it can be supplied in a practical manner, is easily seen. It will introduce improvements into the creameries similar to those introduced into breweries by means of the study of yeasts. In normal butter making as practiced to-day, there is no way of obtaining any control of the bacteria present in the cream. A given specimen of cream will contain a large variety of bacteria. Conn [90] has shown that there may be a score of different species of bacteria growing in cream which has been collected in the normal manner. The butter maker has no means of regulating this assortment or even of knowing anything about it, but must depend upon what has been brought to him. During the ripening process there will ensue a conflict of the different organisms with each other, and the result will depend upon a variety of circumstances. The result will be influenced by temperature, variety of species, quality of the cream, and length of time of ripening, as well as by the advantage which certain species of organisms may get from an earlier start. In such a conflict it will be a matter of accident if the proper species succeeds in growing rapidly enough to produce its own effect on the cream unhindered by the others. Now it certainly makes a great difference in the product which species of bacteria happen to grow most rapidly. Storeh found only a single species that produced the proper aroma and Conn has found that cream ripened with improper species of bacteria produces very poor butter.

The proper aroma of butter is a very intangible matter for study. It is not due to the volatile acids, as was formerly supposed, for the butter aroma has been found to be produced in solutions containing only milk sugar and peptone. Evidently this aroma is in some way connected with the first products of decomposition which are set up in the cream as the result of bacteria growth. But these decomposition products are very numerous and not all desirable. The bacteria which grow in ripening cream have been found to produce all sorts of disagreeable flavors and tastes in milk or cream if allowed to act unhindered. It seems to be only the first products of the decomposition that have the pleasant flavor, the later stages of the decomposition giving rise to products of a very different character. Too long a ripening results in the production of a butter containing too strong flavors, and one of the difficulties of butter makers is to determine the right length of time for proper ripening. Indeed the greatest difficulty which the butter maker has to meet is in obtaining a uniform product. Proceeding according to rules which his experience has taught him, he can usually obtain a good product. But even the best butter makers will sometimes fail from causes not explained.

Now, while the trouble is of course not entirely due to difficulties in the ripening, there is no question that this is one of the prominent sources of difficulty. The butter maker can have no certainty that his cream is supplied with the proper species of bacteria to produce the proper aroma, and can never be sure as to the result of the ripening. If he could be furnished with a ferment for the purpose, as the brewer is furnished with yeast, one of his chief difficulties would be overcome. It is in this direction that experiments are being directed to-day. The bacteriologists have offered the butter maker in Europe even now a ferment with which to ripen his cream, and by using fresh milk and separating the cream with the centrifugal machine, there is nothing in the way of ripening cream with the ferment offered, unhindered by the other organisms which are usually present. But the work as yet is only preliminary. While there has been found a species of bacteria which produces a good result, we do not yet know enough of the effect of the ordinary species of bacteria. We have no knowledge as to whether more than a single species can produce good results, nor do we know whether the same species are used in creameries in this country and in Europe.

The matter of the production of the proper butter aroma as the result of the use of artificial ferments in ripening cream, is at present too uncertain for definite conclusions. We may be confident that the flavor of the butter is largely dependent upon the decomposition products of the bacteria that grow in the cream, and we have positive evidence that some organisms will produce much better quality of butter than others. We may hope that the further study of the decomposition products of different organisms and their relation to cream and butter will offer to

the butter maker the solution of this difficult problem in the future. If that occurs, we may hope not that the butter maker will be able to make better butter than the best that is made to-day, but that he will be able to obtain the best product with uniformity; and we may also expect that the creameries which at present make an inferior quality of butter will be able to improve it so as to compete with the best.

As for the other purposes of ripening, it is not possible to say much at present. Evidently the greater ease of churning and the larger product obtained from ripened cream are matters closely related to each other. The simple fact is that fat is more easily collected into masses of sufficient size to be removed mechanically from the butter-milk. But why the ripening makes them thus more easily collected, is not yet fully explained. The difficulty of an explanation lies in the fact that we do not know exactly the condition of the fat in the milk. The old belief that each globule was surrounded by an albuminous envelope has been disproved. But it seems certain that the fat does not lie freely in the milk serum. The most recent suggestion is that of Babcock [89], who thinks that there is formed in the milk shortly after it is drawn a minute amount of fibrin like the fibrin of the blood. Just as this fibrin in the blood entangles the corpuscles and produces the clotting, so here it produces a similar clotting, but owing to its extreme minuteness in quantity the clotting is not noticeable. If this fibrin is really formed in this manner, it may be that it entangles the fat globules and prevents their ready fusion. Upon this supposition the effect of ripening in rendering the churning more easy, is explained by the partial solution and digestion of this fibrin material, by the solvent power of bacteria growth [Conn 89].

The treatment of cream for butter making needs, therefore, to be very different from its treatment for ordinary purposes. The milkman desires his milk to be as free from microorganisms as possible, but the butter maker uses them and takes pains to cultivate them, but he wants the proper species if the bacteriologists can furnish it to him. He desires their action on the albuminoids of the milk, which renders the churning easier for him, and he desires still more the early products of their decomposition, which give him the desirable butter aroma.

CHEESE MAKING.

If bacteria are desirable allies of the butter maker, they are absolute necessities to the cheese manufacturer. Without their agency in ripening cream the butter may taste flat, but it is still usable, but cheese is worthless without them. New cheese is not palatable; it tastes like fresh milk curd and is not at all pleasant. The proper flavor of cheese appears only as the result of a ripening process which is allowed to continue for several weeks or months, the flavor slowly growing stronger all the while.

The ripening of cheese has been conclusively proved to be a matter of the action of microörganisms. Cohn [75] first found bacteria in cheese, stating that *Bacterium lactis* was especially abundant. But it was Duclaux who first connected the ripening with the growth of these organisms. His first paper [77] gave the results of a chemical study of the ripening process and showed that it consisted chiefly in the transformation of insoluble casein into soluble albuminoids and that the process was associated with the production of several ferments. Three years later [80] he made a study of the bacteria in such cheese, and determined that they were very numerous and comprised several species. Some of them were aërobic while others were anaërobic. During the ripening there were produced several gases—carbonic acid, hydrogen, and sulphureted hydrogen, and a large number of decomposition products, such as alcohol, oxalic acid, carbonate of ammonium, leucin, tyrosin, etc. In general the process was quite similar to the digestion by the digestive fluids of the stomach and alimentary canal. At this time Duclaux first suggested that certain of the bacteria produced ferments as the result of their growth similar in their characters to the digestive ferments, a discovery which we have seen to be so well established by later work.

The ripening of cheese was now studied by others, Schaffer, Bondzynski, and Benecke all confirming Duclaux. Schaffer prevented the growth of bacteria by subjecting the cheese to a stream of carbonic acid and found that under these conditions the cheese would not ripen. Benecke [87] concluded that the species *Bacillus subtilis* was the chief organism concerned in the ripening process, although others were found with it. In a later work Duclaux [87] applies modern methods of bacteriological study to the subject and found seven species of aërobic organisms and three species of anaërobic organisms present in the cheese. He regarded them all as concerned in the process. The ripening, he said, concerned chiefly the casein, and was due to the combined effect of all of the bacteria present, each aiding the others, and each having a share in the decomposition of the casein. The aërobic organisms acted at the surface and the anaërobic organisms acted in the interior, and thus the whole cheese becomes thoroughly ripened.

The most systematic work was done, however, by Adametz [89]. This observer proved that the ripening was due to bacteria growth by treating fresh cheese with a disinfecting agent, which would prevent bacteria growth without affecting the chemical condition of the cheese. Under these conditions the cheese did not ripen. He also made quantitative estimates of the number of organisms present, finding from 850,000 to 5,600,000 per gram, and this number was found to increase slowly during the ripening process. He also tried to determine whether the ripening was due to the combined action of many species of organisms or to a single species. For this purpose he studied many specimens, and studied the cheese at intervals during the ripening. He found many species of bacteria present, but as the ripening went on

one species was found to increase at the expense of the others, and was much more abundant at the close of the ripening than any of the others. This species he always found while the others were more variable, and hence he concluded that this species was the cause of the ripening. The organism in question was not *Bacillus subtilis*, as had been supposed by Benecke, but a species to which no name has been given.

Shortly after this Freudenreich [90] carried on a set of experiments of a similar import to those of Adametz, confirming his results. He obtained rather large numbers of organisms in his cheese, but agreed with Adametz that the ripening was due to a single species of organism rather than to the combined action of a large number.

At this point the knowledge of the normal ripening of cheese rests at the present time. But few observations have been made in regard to abnormal ripening. The greatest difficulty that the cheese manufacturer has to contend with lies in this direction. He can not be sure of a uniform product. In spite of all precautions his cheese will sometimes undergo abnormal troubles and become worthless by changes taking place during the ripening process. These troubles have been attributed to every sort of difficulty, including health and condition of the cow, the condition of the barn, the food of the cow, etc. In some cases they have actually been traced to filth connected with the management of the cows. Recent experiments have indicated that the direct result is in all cases to be attributed to the action of abnormal species of microorganisms which get into the milk, and hence have a share in the ripening of the cheese. Certain it is that black cheese, bitter cheese, and cheese flecked with red spots are all thus caused, and several other troublesome infections have with certainty been traced to microorganisms. Freudenreich [90] has experimentally shown that if milk is inoculated with certain species of bacteria which should not be present and the milk is then made into cheese, the cheese will ripen in an abnormal manner and become worthless, while the control cheese is perfectly good. According to Adametz [91] either bacteria, yeasts, or molds may be the cause of the abnormal ripening of cheese under different conditions. But while abnormal ripening is undoubtedly due to growth of improper species of organisms, we can not at present determine how far the variations in the ripening are due to different species of organisms planted in the curd and how far to different conditions of the ripening. Each doubtless has its effect, and much further study is needed in this direction.

It is evident that the presence of bacteria in cheese is inevitable. The milk from which it was made always contains them, and when made into cheese part of the bacteria at least will be inclosed in the cheese. Here they find proper conditions for growth. The conditions are not very favorable, it is true, for the density of the cheese prevents ready access of air, and the aërobic organisms suffer in consequence except at the surface. The lack of moisture is also doubtless a disadvantage. But in spite of these disadvantages the bacteria grow slowly,

and soon produce profound chemical changes. They give rise to the peptonizing ferment, which acts upon the casein, rendering it partly soluble. Besides this, they induce numerous other decomposition changes, the total result of which is the production of the rich, delicately flavored cheese for the market. But though many chemical studies have been made of ripening cheese, we are not in condition at the present time to follow the process beyond stating the few salient facts already mentioned. The cheese maker thus forces the bacteria to give him products for which he obtains a high price. Of course so far as the food value of cheese is concerned, it is the casein and the fat which render cheese valuable, but its market price depends not upon the quantity of casein, but upon the flavor, and this flavor is supplied by microorganisms. To a certain extent also it is true that the different flavors of different cheeses are due to the action of different species of organisms in the ripening, although we know little in regard to this matter at the present time.

What the practical application of these results will be in the future, it is impossible to say. We have as yet only learned that there is a causal connection between the ripening and the microorganisms, but the conditions affecting their growth, the variety of species which can produce a normal ripening of cheese, whether different species of organisms will produce different flavored cheeses, whether the cheeses of the markets are due to different organisms used in the ripening or chiefly to different conditions under which they are grown, are all problems to be settled before any practical results can be expected. But we can confidently predict one result: If we ever do succeed in reducing the ripening of cheese to a systematic process and are able to use the proper species of organisms to produce it, we may expect an end of the cases of poisonous cheese of which so many instances are on record. The poisons in these cheeses are due to the growth of mischievous organisms, and will be avoided when we learn to ripen cheese with pure cultures of the proper species of bacteria.

We may then perhaps predict a time in the not distant future when both the butter maker and cheese maker will make use of fresh milk. The butter maker will separate the cream by the centrifugal machine in as fresh a condition as possible and will add to the cream an artificial ferment consisting of a pure culture of the proper bacteria, and then ripen his cream in the normal manner. The result will be uniformity. The cheese maker will in like manner inoculate fresh milk with an artificial ferment, and thus be able to control his product. Perhaps he will have a large variety of such ferments, each of which will produce for him a definite quality of cheese. To the dairy interest, therefore, the bacteriologist holds out the hope of uniformity. The time will come when the butter maker will always make good butter, and the cheese maker will be able in all cases to obtain exactly the kind of ripening that he desires.

LIST OF REFERENCES TO THE LITERATURE.

In the following list the author and the year (referred to by numbers in brackets in the text) and place of publication are given for each article cited. Where an author has published more than a single article in the same year the subject of each article is indicated.

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Miscellaneous Bulletins.—No. 1, Proceedings of Knoxville Convention of Association of Agricultural Colleges and Stations, January, 1889; No. 2, Proceedings of Washington Convention of the Association, November, 1889; No. 3, Proceedings of Champaign Convention of the Association, November, 1890.

The Experiment Station Record, vol. I, 6 numbers; vol. II, 12 numbers; vol. III, Nos. 1-10. Copies of the station and Department publications abstracted in the Record can, in many instances, be obtained on application.

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